"In der kleinsten Zelle - da liegen schon alle Räthsel des Lebens vor uns und bei der Erforschung der kleinsten Zelle - da sind wir mit den bisherigen Hilfsmitteln bereits an der Grenze angelangt."

Studies in the Chemistry of the Animal Cell especially with reference to the Nucleins and Paranucleins.

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B.Sc. (1892)
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Handed in as Thesis for M.D. degree in Edinburgh University
April 29. 96

Studies in the Chemistry of the Animal Cell.

Especially with reference to the Nucleins and Paranucleins.

In the life of the cell its development, maintenance and decay, we see the life of the organism in miniature reflected. In its interior the most manifold physiological processes play their parts and not, at least to the same extent, in the fluids which surround the cell. The extent of the chemical processes and the alteration from one chemical act to another are due to the working of the cell. We may study the cell from two great standpoints, first the histological, secondly the chemical. That these two modes of study are not completely divided from one another is seen even after a superficial study. For the histological side depends largely on the chemical for its existence e.g. with regard to the investigation of the effects of different staining re-agents on various tissues, one has to deal with the effect of one chemical agent upon another. The more

Modes of Study

Micro- and Macro-Chemical Reactions

intimate our knowledge of the chemistry of the tissues of the organism, the more likely are we to find out new methods (staining) to bring the different parts of these tissues to light. From the other side, namely the study originally of the histological, one is brought face to face with a series of discoveries in which the effect of different chemical agents on special parts of each tissue is of a definite fixed character and for which there must be some reason from the chemical side. That some acid staining re-agents only re-act on alkaline tissues and some alkaline re-agents only on acid ones makes one suspect that the combination in either case must be of the character of a salt. That some of the constituents of the animal organism at one time re-act acid and at another alkaline affords an explanation for the fact that in many cases both alkaline and acid re-agents are able to fix one particular constituent of the tissues. From our knowledge of chemistry we are able to devise methods which bring out the different cellular constituents in the form of easily recognisable and characteristic stains. Staining methods to detect

presence of Iron, Sulphur, Phosphorus, Oxygen, in the nascent state are examples from the side of the elements. During the last year I have been occupied with the subject from the chemical side and more especially with regard to the chemistry of the nuclei. The great obstacle ~~of~~ to a thorough knowledge of the cell is the fact that the tissues when examined chemically are not in the living state, and we can only draw our conclusions as to the composition of the cell from an examination of the same ~~in~~ as obtained after death from fluids or tissues rich in them. We may examine the ~~subject~~ cell either in such fluids as the blood or pus or in richly cellular tissues as those of Thymus, Testis, Spleen, etc. The tissues mentioned are also suitable media for the investigation of the nuclear chemistry. In the case of the blood, one must go to that of different birds, e.g. goose, duck, hen, in order to obtain nuclei in the red blood corpuscles of sufficient size to enable one to make a chemical examination. IN the earliest periods of existence, the different cells show a similarity which gradually disappears as the age advances. There are certain constituents which are present in every actively growing cell and those

Primary Cell Constituents and Secondary Cell Constituents

Kossel[X] has named the Primary constituents, while there are others which do not appear in every growing cell and those the same author has termed Secondary. Among the primary are included, Albumines, Nucleo-Proteids, Nucleins, Lecithin, Glycogen, Cholesterin, Water and Salts: as Secondary, Fat, Glycogen + Pigments. It is extremely difficult to know ~~wkkar~~ whether the Primary constituents are necessary for the life of the cell or whether they are only products from the life processes which take place in the cell. When we come to the question as to which belong to the protoplasma and which to the nuclei we are met with a still greater difficulty. From a comparative study of cells rich in nuclei compared to those with only a small amount of nuclear substance we are able to notice many differences in the chemical composition of ~~either~~ both. The older view that the protoplasma of the cell consists most largely of Albumines or Globulins is not now held. In all probability the latter at least are present to a small ~~extx~~ extent either as food substances or decomposition products in the protoplasm of all cells; but the great mass of the cells seems to consist of more complicated

Nuclear + Protoplasmic Constituents.

[X] Behrens Kossel u. Schiefferdecker Bd. I u II. For Literature references see end of Bd I pp. 266-269 of paper.

Proteids
/
Nucleo-Proteids
Glyko - "
Haemoglobin
Series

Nucleo-Proteids.

bodies. These complicated bodies, namely Proteids, may be divided into at least three classes, firstly Nucleo-Proteids or Proteids which on decomposition furnish an albuminous part and a Nuclein radicle, secondly Glyco-Proteids or those which furnish, in addition to the Albumin, a Carbo-hydrate, and thirdly those in which Iron, Sulphur and rarely Copper enter into the proteid molecule as in the Haemoglobin series. With the members of the first class I shall have most to say in this paper. In the first place, let me give a short account of our present knowledge of the group. Hoppe-Seyler and Miescher (x) were the first to shed a light upon the structure of the nucleus from the chemical point of view. They extracted from the nuclei of the pus cells a body which they termed Nuclein because it formed the chief constituent of the nuclei of these cells. Later on, in the plant as in the animal kingdom, different workers have succeeded in isolating similar rich phosphorus holding bodies. The knowledge, however, of these bodies was of an extremely limited nature until comparatively recently when the work of Miescher, Lubavin, Altmann, Hammarsten but most of all

(x) For all Literature References See end of Paper.

Kossel and his pupils has shed a brighter light upon the whole. Although even at present one cannot definitely grouptogether all the different nuclear combinations in an exact scheme, still we are able to give a general idea of the probable way in which the Nucleo-Proteids are built up and are split up. We may first of all regard the true Nucleim as the characteristic constitutent of the nucleus. In its molecule the Albumin and the Nucleic Acid are combined. This so-called Nucleic Acid was first prepared by Miescher out of the spermatozoa of the salmon, but its chemical nature was not discovered by him. He termed this acid a Nuclein and after analyses gave as its characteristics the proportion between Nitrogen and Phosphorus (3:1) and the high percentage of the latter (namely over 9 per cent.). He gave as its formula $C_{29} H_{49} N_9 P_3 O_{22}$ Altmann and Kossel recognised the nature of this body, at least so far as we at present know it, and the former termed it Nucleic Acid. At the same time, he laid stress upon the power which the acid possessed of precipitating Proteids and Albumoses in Acetic Acid fluids and suspected that these precipitates were true artificial Nucleins. If a true Nuclein be combined with a more

or less Proteid detachable from it by Pepsin, the combination is termed a Nucleo-Proteid. A Nuclein or Nucleo-Proteid in the first place always contains a high percentage of Phosphorus (4-6%, leaves after peptic digestion (slowly digestible - see later on in this paper) an indigestible (so far as our knowledge goes) remainder and on decomposition from the action of Sulphuric Acid in the heat furnishes Nuclein bases. The albumin may either be firmly or loosely combined with the Nucleic acid or the latter may even occur in the free state, as in the spermatozoa of the salmon. We term a Nuclein which, after treatment with boiling concentrated Barium Hydrate, furnishes no Nucleic Acid a firmly combined Nuclein, that is to say, Baryta is not able to split the nucleic Acid from the Albumin component of the Nuclein Molecule ~~into its two constituents~~, but it leaves the combination as before. Naturally there may be other ways in which we may split up the Nuclein Molecule into its two constituents, but up to the present we do not know them. During the course of this paper I shall have to revert to this again, as the method has been exceedingly often employed by me in order to test the fixity of the combination between the constituents in the Nuclein Molecule. I have mentioned that Nucleic

Acid exsists in the free state in the spermatozoa of the salmon. In the loosely combined state, it exsists in the Nuclein of the Thymus gland of the calf. In the firmly combined state, it exsists in the Nuclein of the Pancreas (Hammarsten), in the Nuclein of the Nuclei of the red blood corpuscles of the Goose, Hen and Duck and probably in most of the Nucleins, exsisting in the adult cell Nucleus of different organs (Spleen, Liver, Brain, Mammary Gland). In addition to the Nucleins and Nucleo-Proteids we have, at least as far as our present knowledge goes, one other class with probably numerous sub-divisions, namely, the Nucleo-Albumins. These are, as in the case of the Nucleo-Proteids, acid bodies which dissolve in weak alkalies, very slightly if at all dissolved by neutral salts, and on digestion with Pepsin leave a body which is only extremely slowly further acted on by the ferment, namely, Para- or Pseudo-Nuclein Ncuelins. This Para- or Pseudo-Nuclein is rich in Phosphorus (2-5%), but unlike the true Nuclein furnishes no Nuclein bases on heating with weak Sulphuric acid. Another distinction between the Nuclein and the Para-Nuclein is this that the acid component of the former is precipitated by H Cl, but not by Acetic

is does not
ld for all
aranucleic
ids as we
all see later.

Acid, while that of the latter is precipitated by the latter acid but not by the former. The acid component of the Para-Nuclein is termed Para-Nucleic acid, but it has only been chemically prepared in the pure state from the Nucleic acid of the Thymus gland as a product of decomposition. Paranucleic acid has not up to the present been prepared from the Para-Nuclein of Casein, Ovovitellin or Ichthyulin. Up to the present I have only spoken of combinations of Nucleic acid and Paranucleic acid with Albumin as constituting a true Nuclein and Para-Nuclein respectively. With these, as they occur in the organism, there is always more or less of detachable Albumin. Further, during the last year or two attention has been drawn to the fact that some of the Nucleins and at least one of the Para-Nucleins on boiling with dilute Sulphuric acid furnish a Cupric Oxide reducing body. Last year Hammarsten obtained such a body from the Nuclein of the Pancreas, but was not able to characterise it definitely. Walter found in the eggs of the female carp a body which possessed a high percentage of Phosphorus, furnished no Nuclein bases, but a Cupric Oxide reducing body. This he termed Ichthyulin (a Pseudo-Nuclein). In all probability many of the

other Nucleins and Para-Nucleins furnish the same. Of very great interest in this respect is the discovery by Kossel of Laevulinic acid in the decomposition products of the Nucleic acid of the same Nuclein of Thymus gland of the calf. According to Tollens,[&] this acid is a sure test for the presence of a Carbo-Hydrate. If this be true, the Cupric Oxide reducing body of Hammarsten's Nuclein may be a decomposition product of the Nucleic acid of the same. Hammarsten's Nucleo-Proteid of the Pancreas showed itself to be a true Nuclein in that it furnished at least one Nuclein base - Guanin - and was ~~only~~ not specially affected by peptic digestion. The Phosphorus percentage was high, namely before digestion 4.5%, after about 5%. The bodes which Halliburton has isolated from numerous organs Thymus, Spleen, Bone Marrow, etc., and which he terms Nucleo-Albumins, have not been at all definitely characterised. The Phosphorus percentages are extremely low and no mention is made as to whether they furnish Nuclein bases or not. While speaking about the decomposition products of the Nucleins and Pseudo-Nucleins respectively, I wish to draw attention to the fact that in all probability

[&] Handbuch der Kohlehydrate Bd. Kap. 1.

Para- or Pseudo-Nuclein of Casein

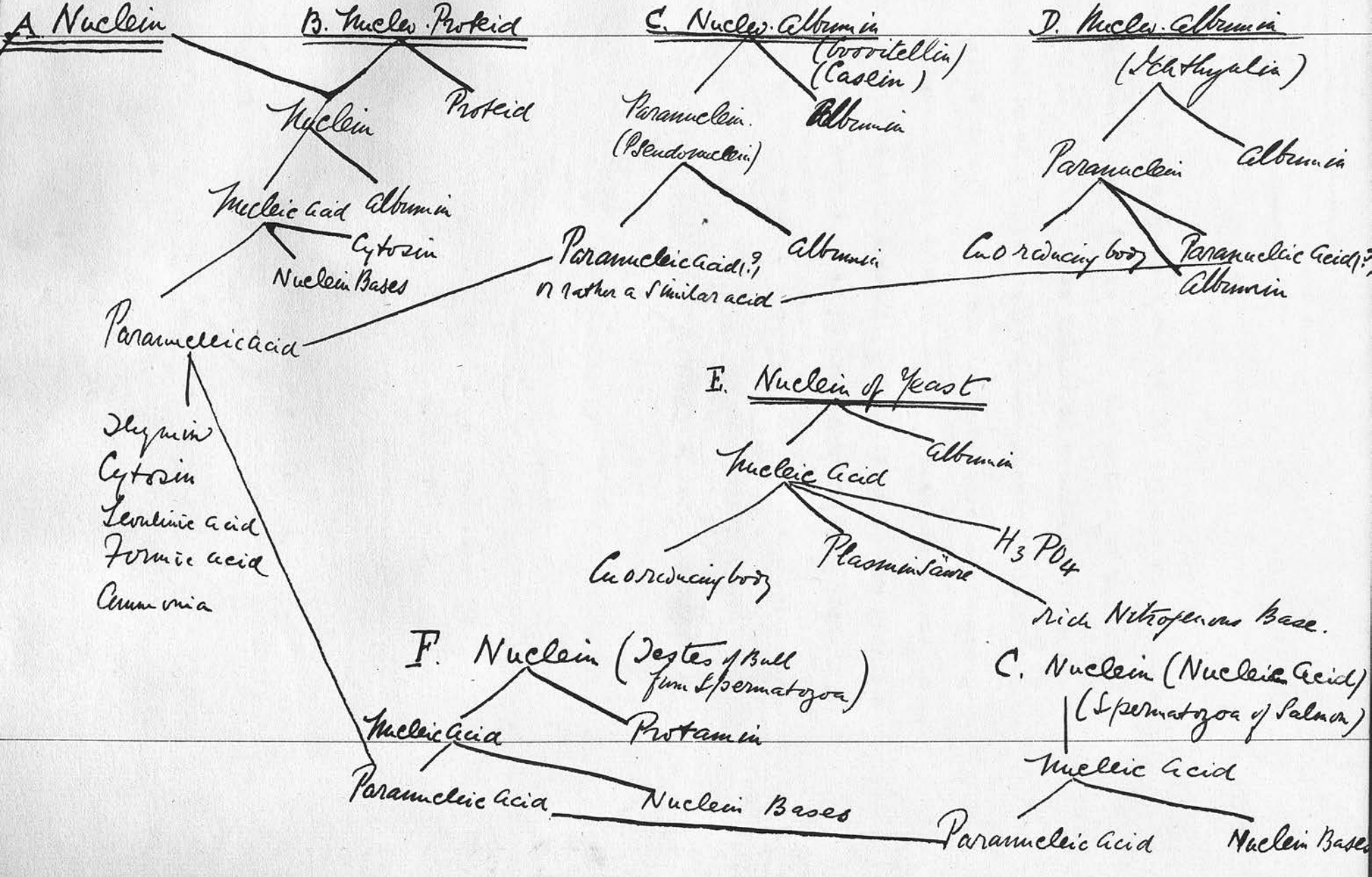
Paranucleic acid has been split off from the para-nuclein Molecule of the Casein. Clara Willdenow (Innaugural Dissertation, Bern 1893) under the direction of Drechsel noted that the Pepsin filtrate from the Casein Para-Nuclein possessed the property of precipitating Egg Albumin in acetic acid solution and that after precipitation of the original filtrate with the acid and refiltration, this latter filtrate does not or only to a slight degree precipitate the Egg Albumin in the same solution. She thought that this might be due to the presence of Nuclein acid. In all probability what she was working with was Paranucleic acid. Salkowski and Hahn, working along the same lines, were also able to note this property, but were unable to isolate the body. What is certain is this, that in the soluble digestive products of the Casein or Casein Para-Nuclein respectively, there is a high percentage of Phosphorus, and, secondly, this high percentage is not due to the presence of free ortho-phosphoric acid or soluble phosphates, but organic phosphorus holding bodies. As up to the present we had no exact knowledge of the way in which the Nuclein is split up if it be split up at all

by the action of the digestive ferments, I have devoted a large part of my work to the solution of this question. The importance of the question as to whether the Nucleins are split at all in the alimentary canal or if they pass through it undigested and unused is manifest from the practical as well as from the purely scientific side. It may be answered in at least two ways, First, by feeding animals who had previously a constant excretion of phosphates in urine, with Para-Nucleins or Nucleins, and observing if there be a sudden rise in the phosphate excretion on the introduction of these bodies into the diet and a sudden fall on their withdrawal. Sandmeyer has answered this question from the side of the Casein Para-Nuclein affirmatively: Gumlich from the side of the Nucleic acid of Thymus and Weintraud from that of the Nucleins of the same gland. In all cases there was an increase of Phosporus excreted in urine immediately after the introduction of the Para-Nuclein and Nuclein respectively and a fall on the withdrawal of the same. These experiments do not show where the splitting up has taken place. They merely show that after the giving of Nucleins or Para-Nucleins there is an increase in the excretion of Phosphorus

in the urine. It may be more definitely answered from the other side, namely, the action of the digestive ferments on the same bodies at body temperature but outside the body. The action of the digestive ferments on the Para-Nuclein has attracted the attention of many workers. This is especially true with regard to the Casein Para-Nuclein. I have already mentioned the works of Willdenow, Salkowski and Hahn. I may also mention Sebelien who investigated the action both of Pepsin and Trypsin on the same body, finding that it was slowly attacked by the former and that the Phosphorus was mostly split up in the form of an organic body (Salkowski and Hahn). The action of the Trypsin goes further, the body being rapidly broken up and the Phosphorus split off in soluble form with the digestive products (Sebelien). Sebelien Bunge found in the yolk of the egg a rich Phosphorus holding body which did not furnish Nuclein bases; but which from its behaviour towards Pepsin and its solubilities in acids and weak alkalis, he regarded as a Para-Nuclein. This body he termed Haematogen from the fact that he regarded it as the forerunner of the Haemoglobin, holding as it does Iron organically bound. He did not examine the nature of the

soluble digestive products after action of Pepsin, nor did he subject the body to the action of Trypsin. Naturally this theory is only a supposition, for we do not know the further changes that take place in the Para-Nuclein of the Ovovitellin. We merely know that it is a rich Phosphorus holding body with a constant though small percentage of organically bound Iron, that it is only with difficulty acted on by Pepsin and that it is a constant constituent of the non-incubated hen's egg. When we dissolve the colourless residue obtained from the ~~yolk~~ yolk of the egg (after ether extraction) in weak hydro-chloric acid (0·25 per cent-) and add to this Pepsin and then keep the mixture at a temperature of 38° to 40° for 24 or more hours, a white precipitate falls in this solution and this precipitate is Bunge's so-called Haematogen (a Pseudo-Nuclein). From this body Altmann says that he obtained Nucleic acid, As I shall show later, this is incorrect, the body being not of the nature of a Nucleic acid, as it does not furnish Nuclein bases. The nature of this body I shall discuss later. From Walter's Ichthyulin Paranucleic acid has never been obtained. The interesting point is, that from this Pseudo-Nuclein, as in the case of

Nuclein and Paranuclein Series.



Hammersten's Nuclein, out of the Nuclein Molecule, a Carbo-Hydrate arises under the action of Sulphuric acid. I may in the following scheme show the relation ship that exists between Nucelo-Proteid, Nucleo-Albumins and Para-Nuclein clearer than can be accomplished simply from description. That the scheme is an imperfect one, I admit, but it is as far as possible correct according to our present-day knowledge.

See Scheme on previous page.

Characteristics of the Nucleins, Paranucleins, Nucleic and Paranucleic acids.

A Nuclein.

1. Is only after prolonged action attacked by Pepsin.
2. Has a percentage of Phosphorus from 4-6.
3. Furnishes on decomposition by acids Nuclein bases.
4. possesses Acid re-action.
5. (X) Consists of a more or less firm combination between Albumin and Nucleic acid.
6. In some cases furnishes a Cupric Oxide reducing body under the action of acids in the heat.
7. Is insoluble (or only slightly so) in weak acids, and is precipitated by different salts, easily soluble in Alkalies.
8. (X) Is split up easily by action of Trypsin and Sodium Carbonate, probably also by action of Bacteria. (This is not the opinion held by previous investigators)

Para-Nucleins.

1. ~~Only~~ With less ~~more~~ difficulty acted upon by Pepsin than the Nucleins.
2. Has a Phosphorus percentage of, in all probability, ~~of~~ from 5-6% when pure.
3. Does not furnish Nuclein bases.

(X) These divisions marked by an asterisk have not been previously worked at and only apply to the Nucleins + Paranucleins examined by myself. There may be exceptions in the form of those Nucleins which have not yet been examined.

4. In some cases furnishes a Cupric Oxide reducing body when acted upon by acids in the heat, from their solutions precipitated by action of Pepsin.

6. Insoluble in acetic acid, slightly in weak inorganic acids.

5. Paranucleic acid is probably split up from the Para-Nuclein by the action of Pepsin; by Trypsin almost certainly.

7. 8. On decomposition with Sulphuric acid in the heat furnishes Laevulinic acid Thymin Ammonia, Formic acid and Phosphoric acid etc

Nucleic Acid.

1. Phosphorus percentage about 9 - 10%.
2. Is a definitely characterised chemical body with the formula $C_{29}H_{49}N_9P_3O_{22}$ (Miescher) $C_{30}H_{52}N_9P_3O_{17}$ (Kossel)
3. Furnishes Nuclein bases on decomposition and the other decomposition products marked under the heading 7 (Para-Nucleins).
4. Is soluble in acetic acid, precipitated by Hydro-Chloric acid especially in the presence of alcohol.
5. Precipitates Albumins, Albumoses & Peptones in acetic and Hydro-Chloric solutions.

Paranucleic Acid.

1. Possesses about 12 percent. Phosphorus.
2. Definitely characterised chemical body with the formula $C_{16}H_{25}N_3$ [illegible] O_{12} (the researches by Kossel on this subject have not yet been published.) Thanks to his courtesy I have been permitted to give the formula here. $C_{16}H_{25}N_3P_2O_{12}$
3. Furnishes ~~more~~ no Nuclein bases.
4. Is precipitated by Acetic acid and dissolved by Hydro-Chloric.
5. Barium salt is soluble in water, precipitable by Alcohol.
6. Precipitates Albumins, Albumoses and Peptones out of an Acetic acid solution- precipitates being soluble at least partially ~~by~~ in dilute Hydro-Chloric acid.

From the Nuclein acid the Paranucleic is easily prepared when the former simply loses the Nuclein bases, the first decomposition ~~is~~ being the Paranucleic acid and ~~no~~ no other product except Cytosin is split off at the same time at water-bath temperature in aqueous solution. Naturally it possesses ~~the~~ a higher Phosphorus percentage than the Nuclein acid.

Cytosin - a rich Nitrogen holding base of strongly basic properties

A. The Nucleic acid has been obtained from the following sources:-

1. In the free state from the Spermatozoa of the salmon.
2. Split up from its combination from Albumin out of
   a. the Thymus Nuclein. (loosely combined)
   b. Yeast.
   c. Spleen.
   d. Red blood Corpuscles of several birds.? (see Kacer, p. 68)
   e. Testes of the Bull.
   f. Herring roe.
   (g. According to Altmann from the yolk of the hen's egg, but this, as I shall show later, is incorrect.)

B. Paranucleic Acid has been obtained only from the Nucleic Acid of the Thymus gland, although in all probability the same body has been obtained from Casein, but not chemically pure.

When we remember how easily the Nucleic acid passes into the form of Paranucleic we must be careful before making positive assertions as to the presence of the one or the other without full proof of the presence or absence of Nuclein bases as decomposition

products. Before leaving the introductory chapter I wish to draw attention to the great importance which these bodies possess with regard to the origin of ~~Sulphuric~~ Uric acid in the body. When we remember the close affinity existing between the Nuclein bases and the Uric Acid, the latter being the highest oxidised member of the series, we are brought face to face with the probable link in the chain between the breaking-down of the Proteid or Nucleo-Proteid Molecule and the origin of Uric Acid. Horbaczweski's experiments (at present being corroborated by Samdmeyer) on the origin of Uric Acid in Mammals in the case of a mixture of ~~Spleen kept~~ Splenic tissue + blood kept in a vessel ~~with blood~~ at body temperature for some days, ~~showing~~ show that when the precautions mentioned by the author are carried out in every detail, no trace of the Nuclein bases is to be found, all being transformed into Uric Acid. If the process of Oxidation ~~being~~ be a shorter one or altered in any of the minor details, Hypoxanthin at once appears and Uric Acid is absent.* Further proofs of the close relationship existing between the Nucleins and Uric Acid are being furnished at present by Sandmeyer, who has obtained Uric Acid from mixtures of blood with not only tissues rich in Nucleins, but from the

Connection between the true Nucleins and Uric Acid

cf. when Arterial blood is allowed to ~~circulate~~ remain in contact with the Splenic tissue sufficiently ~~long~~ long.

cf. in diseased conditions of the blood.

* Horbaczewsky. Monatshefte für Chemie Bd 10 1889 S. 624.
" Sitzungsbericht d. Wiener Akad. 1891 III S. 78-132
Giacosa Du Bois-Reymond's Archiv. 1893 S. 109
Wien. Med. Blätter 1890 No 32

Nucleins and also Nucleic Acid, when the two latter are placed under the same conditions as was the case ~~with~~ in the experiments of Horbacweski. And now at the present day we have the further interesting fact of the presence of the Carbo-Hydrate Molecule in at least some of the Nucleins and Para-Nucleins ~~and~~ shown by the presence of Laevulinic Acid in the decomposition products from the Thymus Nucleic Acid. The difference in the composition of the hen's egg before and after the appearance of the Embryo shows the close relationship existing between the Para-Nucleins and the true Nucleins. From the former no Nuclein bases are to be obtained, while from the latter they can be easily prepared. From the undeveloped eggs of Bombrix mori L. (silk spinner) less than 0·02 per cent. Hypozanthin, Guanin (Adenin) were obtained and only traces of Xanthin. The reason even for this small quantity is that there is always a certain amount of Nuclear Nucleins present, while the great mass of the "Dotterzellen" are made up of ~~two~~ true Nuclei. From the developed eggs after 13 days incubation at 23° C. 0·21 per cent. Nuclein bases were obtained. The developed eggs contained also much less ~~Nxkxogex~~ Glycogen. The more one enters into this subject, the study of the cell

(Dichomiroff. Chem. Studien über Entwicklung der Insecteneier) Zeit. f. phys. Chem. Bd. IX p. 518, 525-529, 532 und 566)

In the case of the Amphibians, Fishes + also in Plants the oxidation of the nitrogenous bases derived from the nucleins does not proceed so far as Uric acid formation. In Birds as is well known the Uric Acid is largely formed from Ammonium Lactate derived from the Proteid molecule a small amount is also formed from the Nucleins of the red cells.

Nucleus, the more ~~are~~ is one we impressed by the fact that here we are dealing with the fundamental life processes. In the egg we find the rich Phosphorus holding Para-Nucleins which do not furnish Nuclein bases transformed into the Nucleins which do. In the milk which the child sucks we find again the Para-Nuclein which does not furnish Nuclein Bases going to ~~from~~ form in all probability the Nucleins which do. In both we find a considerable percentage of Iron. As the child (respectively the cell) grows we find a gradual transformation taking place, a continual increase in the cellular elements with their Nuclei and at the period of full growth a firm combination between the Nucleic Acid and the Albumin in the Nucleus. One other constituent of the cell I have still to speak of as closely related at least to the Nucleic Acid and that is a base, termed Protamin. This body was first discovered by Miescher in the salmon Spermatozoa. He regarded it as a combination with a Nuclein which he also prepared from the same source. This Nuclein was shown by Kossel to be in reality a Nucleic Acid and the latter author regards the Protamin - Nucleic Acid relationship to be that of base to acid - a salt. Protamin Platinum Chloride

(Maly's Jahresbericht. Bd. 4. s 355) $PtCl_4$ 2(HCl. $C_8H_{16}N_{4,5}O_2$) was analysed by Piccard. It also forms compounds with Hydro-Chloric and Nitric Acids which are, even as the base itself, soluble easily in water, with more difficulty in Alcohol ~~in~~ & Ether. The base is insoluble in Alcohol and Ether. The base is precipitated by Nitrate ~~Nitrate~~ of Silver and the other precipitants of bases. According to Mieshcer the Spermatozoa of the salmon contains in 1000 parts :-

Nuclein (Nucleic Acid)	487 parts
Protamin	286 "
Albumins	103 "
Lecithin	75 "
Cholesterin	22 "
Fats	45 "

Kossel also believes that there is a special Nucleic Acid from which ~~its~~ each Nuclein base is obtained e.g. an Adenyl Säure, Guanin Säure etc. The body called Spermatin has not been yet definitely characterised, but it seems to be of the nature of an ~~A~~ alkali-albuminate. Within the last two years, while Siegfried ~~has~~ was ~~Author~~ investigating the chemical nature of the flesh extractives, he discovered a new organic Phorphorus Acid which he has termed Phosphorfleischsäure

Phosphor-fleisch-säure or Paranuc-leone.

Why I mention this body here is because of the probable close relationship existing between the same and the members of the Nuclein series. The Phosphorfleischsäure is an acid obtained from Proteid-free extracts from the muscle substance. The solutions of the extractives of the muscle possess to a high degree the faculty of reducing. In all probability the reducing substances of the tissues possess aldehyde character. From the extract obtained from many kilogrammes of horse flesh, the author, after removing the known constituents by the usual methods and completely precipitating with Phosphorfungstic Acid obtained a N-holding substance which reduced an ammoniacal solution of silver Nitrate. The phosphorfleischsäure through the action of Barium Hydrate at a temperature of 50° furnishes Fleischsäure along with Succinic, Lactic Acid, Phosphoric Acid. From the Phosphorfleischsäure of the muscle extract Fleishsäure and some of the decomposition products and of the Carbo-Hydrates and Phosphoric Acid are split off. In an earlier stage of a decomposition the Phosphorus is present in an organic form. From the study of the decomposition products of the Nuclein and Para-Nuclein series and those of the Phosphorfleisch säure, the hypothesis formulated by Siegfried that the latter belong to a

products

very closely allied group becomes a probable one. This acid possesses the faculty of keeping otherwise insoluble salts in solution e.g. Calcium and Magnesium. The importanee of such a body as Carrier of Calcium Salts in solution is apparent at first sight. As Siegfried has shown, the Antipeptone of Kühne is in reality Fleischsäure. Because of this Siegfried proposes the name Paranucleone for the latter body in its combination as Phosphorfleischsäure. The other name that he proposed was Muscle Nucleon.

From the study of this introduction, giving a general view of the present day knowledge of the chemistry of the Nucleins and allied bodies in animal organism one notes very frequently points which still require clearing up. Allow me shortly to sum up some of those points. First of all, the nature of the body as precipitated by Nucleic Acid from Proteid solutions is not yet known. Are they similar to the Nucleins which have been prepared from the Nuclei of different cells? Are they firm combinations between Nucleic Acid and Proteid or loose ones? If they show marked similarity to the true Nucleins as existing in the cell, the Liebermann hypothesis with regard to the part

which Metaphosphoric acids is supposed to play in the Nuclein Molecule has received another blow.

In order to settle this point I have investigated those artificially prepared Nucleins in different ways and have shown points of similarity to ~~both~~ the natural Nucleins.

Secondly, our knowledge of the nature of the natural Nucleins is almost limited to two or three members of the class and no one member has been fully investigated in the manner necessary to fix definitely its nature e.g. characters of the purely prepared Nucleins

1 resistance to Pepsin and Trypsin

2. Fixity of combination of Nucleic Acid and Proteid.

3. Nature of the combination.

4. Nature of the products split off by digestion of the Nuclein.

The other points which still require clearing up I shall mention later on in this paper.

I may mention however that as our knowledge of the Paranucleins is so limited, Ihave tried to fill up some of the blanks by investigating the so-called Paranuclein of Ovovitellin and also by preparing Paranucleins synthetically.

Section 11.

The Nature of the Artificially prepared Nucleins.

Syntheses of the true Nucleins.

Nucleic Acid shares with metaphoaphoric acid and Paranucleic Acid the power of precipitating Proteids, Albumoses and Peptones from their acid solutions. The Nucleic Acid can do this either in fluids ~~precipitated~~ acidified with acetic or Hydro-Chloric ~~Acid~~ Acids. The Para-Nucleic can only do this in the presence of the former acids. In order to test in the first place the nature of the artifical true Nucleins it was necessary to prepare a Nucleic Acid free from Albumin. This was done according to the Kossel-Neumann method. That is to say fresh Thymus glands from the calf were finely hacked after being freed from blood-vessels, fat, etc. The finely minced tissues were then placed in a large vessel with about equal quantities of distilled water to which a few drops of chloroform were added. This was allowed to stand over night and, after the mixture had been thoroughly shaken up and stirred for the first few hours. The mixture was on the following morning filtered through gauze and the pink cloudy fluid obtained treated in

Preparation of Nucleic Acid.

the following way:- a saturated solution of Barium Hydrate was prepared (one part of the powder to three of boiling water) and this was added to the pink fluid the mixture being stirred all the time. A thick pinkish precipitate falls slowly and settles on bottom of vessel. This is allowed to stand until upper part of fluid is absolutely clear and when this has occurred the fluid is siphoned off and the precipitate poured on a folded filter. After the precipitate has been well dried, it is then washed with a cold saturated Barium solution. The precipitate is then carefully scraped from the filter, divided finely up in a small quantity of water, then neutralised ~~utilised~~ with weak Acetic Acid and in conclusion made slightly acid by the same. The mixture, on the addition of more water, is poured into a large flask of about 6 litres capacity and placed on a paraffin bath where it is kept at boiling point for two hours. Connected with the flask is an upright Liebig's condenser so placed in order to keep the bulk of the fluid approximately constant. ~~(I have sketched on the opposite page roughly the nature of the apparatus).~~ The flask, on the conclusion of the boiling, was removed carefully and the fluid poured through while

still hot, and filtered in the way mentioned above. The residue is then subjected again to the process of boiling with a new quantity of water and again filtered until all the Nucleic Acid ~~was~~ is extracted from the gland. The collected filtrates were poured into a mixture of Alcohol and Hydro-Chloric Acid in the proportions mentioned in the original paper. A fine white flocculent precipitate of Nucleic Acid falls slowly to the bottom of the vessel. This is treated in the way described by Kossel in order to obtain the acid Proteid free. The Nucleic Acid is now tested to see if it be free from Barium and Albumin. ~~In~~ A one per cent. solution of the acid was prepared. The albumin employed at first to produce precipitates was a one per cent. solution of Syntonin in the Hydro-Chloric Acid prepared in the usual way. Now the further treatment occurred in one of two ways, either in adding the Syntonin to the Nucleic Acid or vice versa until no more precipitate appeared. Immediately on the Nucleic Acid and Syntoninsolutions coming together a thick pure white flocculent precipitate appeared which rapidly settled at the bottom of the vessel. The supernatant fluid was removed and more Syntonin added to it until no more precipitate appeared. The

Preparation of the Syntonin Nuclein.

collected precipitates were stirred up in distilled water containing a few drops of Hydro-Chloric acid, filtered rapidly and washed in distilled water, alcohol and Ether. The body at the end re-acted acid, was easily dissolved in alkalies, (ammonia or soda) and was of a pure white colour. One portion was dried at 110° until of constant weight and the Phosphorus present estimated according to Weibull's method that is, incineration by Kjeldahl's method, and precipitation of the Phosphorus by Ammonium Molybdate, if either Iron or Calcium were present; by Magnesia mixture alone, if the solution were free from Iron, and the precipitates treated in the usual way, that is, the Ammonium Magnesium Phosphate incinerated and the Phosphorus weighed as Magnesium Pyrophosphate. From this, the Phosphorus percentage was reckoned. Another portion of the precipitated body after purification in the way described above, but without heating at 110°, was subjected to the action of Pepsin for shorter or longer periods at a temperature of 38°. The undigested remainder was filtered from the fluid, washed with distilled water, finally with Alcohol and Ether and dried to a constant weight

as de
Free

Action of Pepsin on this Nuclein

at 110°. The dried residue was then incinerated by the same method and the Phosphorus estimated. In this way we obtained the Phosphorsu percentage of the precipitated body before and after the action of Pepsin. I shall give now in detail a few of ~~the~~ a series of numerous experiments.

Example 1.

1·988 grammes Nucleic Acid was dissolved in 200 c.c. boiling water (the Bunsen burner being immediately removed on the addition of the Nucleic Acid in order that the transformation into Para-Nucleic Acid ~~may~~ might as far as possible be hindered). After the solution had cooled, it was rapidly filtered through a folded filter paper. To this solution a Syntinin one of 0·25 per cent. was added until no more precipitate appeared in the mixture. The accumulated precipitates were rapidly filtered from the solution, washed with a~~s~~cidulated water (HCl). then with distilled water and finally with Alcohol and Ether. The pure white precipitate obtained in this way was divided in two portions, the first of which after drying was incinerated and the Phosphrus estimated in the way mentioned above. The other portion was

digested with Pepsin for 10 hours at 38°.

Analyses.

I. (1st undigested portion)

0·882 g. Nuclein gave 0·1886 g. $Mg_2P_2O_7$

corresponding to 0·0327 g. P.

i.e. 3·707 per cent. P.

II. 2nd Portion with 100 c.c. Pepsin ~~Hyd~~ HCl

10 hours digested.

0·469 g. Nuclein gave 0·0675 g. Mg P O

corresponding to 0·0188 g. P.

i.e. 4·088 per cent. P.

The reason for the higher Phosphorus percentage in No. 2 is that in the case of the first portion in addition to two Nuclein there was also present a small quantity of coagulated Albumin, so that the first portion represents a Nucleo-Proteid, the second a true Nuclein.

Example 2.

2·516 g. Nucleic Acid was dissolved in 250 c.c. boiling water, the solution allowed to cool and then precipitated with a Syntonin solution and the precipitate treated as explained under Example 1.

Analyses.

(1) 0·57 g. Nuclein gave 0·084 g. $Mg_2P_2O_7$ corresponding to 0·0232 g. P = 4·07 per cent. Phosphorus.

(2) Second portion digested with 100 c.c. Pepsin Hydro-Chloric Acid (prepared from pig's stomach) 12 hours at 38°

0·4717 g. Nuclein gave 0·0692 g. $Mg_2P_2O_7$ corresponding to 0·019 g. P. = 4·027 per cent. P.

In this example the Nuclein before and after Peptic digestion contains practically the same amount of Phosphorus that is to say the body is a true Nuclein.

Example 3.

2 g. Nucleic Acid were dissolved in 300 c.c. boiling water and precipitated in the usual way by Syntonin. The precipitate was purified in the way previously described and divided into two portions.

(a) 1st portion 0·751 gave 0·112 g. $Mg_2P_2O_7$ corresponding to 0·031 g. P = 4·127 per cent. P.

(b) 2nd portion was digested with 110 c.c. Pepsin HCl (from pig's stomach) 13 hours at 38°.

0·294 g. Nuclein gave 0·037 g. $Mg_2P_2O_7$ corresponding to 0·01 g. P equals 3·401 per cent. P.

In this case there has been a ~~least~~ loss of Phosphorus from the prolonged or excessive action of the Pepsin solution.

Example 4.

5 g. Nucleic Acid were dissolved in about 200 c.c. of a 15% solution of Natrium Acetate in the warmth (on water bath). ~~When~~ Then cold Syntonin solution was added until no more precipitate appeared in the mixture. The solution was then filtered and the precipitate after being treated in the usual way was analysed.

Portion 1. 0·893 g. Nuclein gave 0·0768 g. $Mg_2P_2O_7$ corresponding to 0·0215 g. P equals 2·408% P.

Portion 2. digested with 100 c.c. Pepsin HCl 8½ hours at 38 gave 0·0455 g. Mg P O corresponding to 0·01258 g. P equals 4·410% P.

Here in the first portion there has been a considerable precipitation of the Syntonin as such by the Natrium Acetate and this has been, in the second portion, removed by the Pepsin with the result that the Phosphorus percentage rises to rather more than the amount present in the other examples.

Portion 1. is an example of a Nucleo-Proteid. Portion 2 of a true Nuclein. Those experiments were repeated very frequently, the results in all cases

being approximately the same. The Phosphorus percentage remained before and after Peptic digestion practically the same, namely about 4%. After prolonged action of the ferment there was a slight loss in the Phosphorus. The action of Pepsin on the Nucleic Acid itself was also investigated.

0·613 g. Nucleic Acid were digested 6 hours with 100 c.c. 0·25% Hydro-Chloric Acid and Marquart's Pepsin. The solution of the acid was not a complete one, although almost so. From the filtrate Nucleic Acid was easily obtained in the usual way. After prolonged action or through the employment of a strong Pepsin solution all the Nucleic Acid was dissolved. The next question was - of what nature is the combination between the Nucleic Acid and Syntonin in the artificial Nuclein? Is the Nucleic Acid firmly or loosely combined? In order to decided this, the following experiment was carried out.

3·734 g. Syntonin Nuclein were dissolved in 300 c.c. in 1% Ammonia solution and the mixture left over night. To this solution was then added a concentrated hot solution of Baryta water (20g. in 60 c.c. water), the precipitate was filtered from the fluid and finely divided up in distilled water acidified

with Acetic Acid and boiled on paraffin bath for 2 hours. The mixture was then filtered while hot and the filtrate, after cooling, poured into 200 c.c. Alcohol to which 30 c.c. concentrated Hydro-Chloric Acid has been added. In this way only traces of Nucleic Acid were obtained. In order totest if Nucleic Acid were present in the filtrate after precipitation with the Baryta water, Alcohol was added to the same and the precipitate which fell to the bottom of the vessel investigated for the presence of Nucleic Acid. In neither case could Nucleic Acid in appreciable quantity be obtained i.e. the combination between Syntonin and Nucleic Acid is a firm one. This result coincides with those obtained from Peptic digestion of the same Nuclein, the ferment being unable to split up the combination. Experiments on the fixity of the combination between the Nucleic Acid and Syntonin were repeated three or four times, but in no case could Nucleic Acid be split off ~~always with negative results~~, even when large quantities of the Nuclein were employed.

As we shall see later on the nature of the combination between the two components in the artificial Syntonin Nuclein corresponds to that exsisting in the natural Nucleins of the Pancreas and of the red blood

corpuscles of birds and not to the Nuclein of the Thymus gland. The fixity of the combination between the components is probably of the greatest importance. As I shall show later on, the Nuclein is gradually though slowly dissolved by the Pepsin, but only to a very slight degree split up by the ferment. The Phosphorus in the filtrate is present in the organic not in the tri-basic form. In order to test whether the solvent action of the Pepsin Hydro-Chloric Acid on the Syntonin solution were due to the action of the ferment or to the Hydro-Chloric Acid, equal quantities of the Nuclein were ~~objected~~ subjected to the action of equal quantities of both agents for similar periods. The Pepsin acted to a much greater extent than the Hydro-Chloric Acid alone dissolved in much more of the Nuclein. The following examples are given to show the state in which the Phosphorus exists in the digested fluids after the action of the ferment.

A. 3 g. Syntonin Nuclein, prepared in the usual way, were digested 15 hours with 100 c.c. Pepsin HCl solution (from pig's stomach). About half of the quantity passed into solution. The filtrate did not precipitate Albumoses or Albumins and did not contain Nucleic Acid. In order to find whether the latter

the latter were actually absent, a hot solution of concentrated Barium Hydrate was added to the filtrate and the precipitate boiled on paraffin bath in acidulated water for 2 hours, and then filtered while hot, allowed to stand over night and next morning poured into Alcohol Hydro-Chloric Acid (for strength see remarks given above). No precipitate of Nucleic Acid appeared. Since about half of the Nuclein has passed into solution, in what form is the Phosphorus present there? We have just seen that it is not a Nucleic Acid and we know that the precentage of Phosphorus after Peptic digestion ~~eben~~ even after long periods is never ~~remarkably~~ appreciably lower, in fact usually shows a slight increase. Naturally there may be a period intermediate where the Phosphorus percentage is a higher one, but in all probability only traces of the Phosphorus existing in the original Nuclein are split off by the action of the Peptic ferment. Then is the Phosphorus present in the organic combinstion or in the ortho-tribasic form. In order to settle this, the Phosphorus in the Pepsin Hydro-Chloric solution before digestion was estimated, first of all that present in ortho form (i.e. the Phosphates precipitable by Magnesia mixture), and secondly the total Phosphorus —the difference giving the organic Phosphorus + that present (if any should be

though that is unlikely) in mono - or di - basic form. Then the Phosphorus is estimated in the two forms after digestion in the filtrates. The increase in the latter case gave the amount of Phosphorus removed by the action of Pepsin from the Syntonin Nuclein. In all cases Weibull's method was employed. Results -

(a) in Pepsin filtrate originally.

1. ortho 0·0061 %
2. total 0·0117%
3. organic 0·056%

B. In Pepsin filtrate after digestion of the Nuclein.

1. Ortho P. 0·0078% P.
2. Total 0·0335%
3. Organic 0·0257%

Here much the larger part namely 92·2% is present in organic form in the Pepsin filtrate.

Example 2. 3·7322 g. Syntonin Nuclein were digested with 100 c.c.solution for 9 hours and filtered Filtrate was examined for Nucleic Acid, but none was present. In the rest of the filtrate the Phosphorus was estimated in the way mentioned above. Pepsin filtrate before digestion contained Phosphorus in the proportions given above.

Pepsin filtrate after digestion of the Syntonin Nuclein :- See next Page

A. Ortho p. 0·0088%

B. Total 0·0454%

C. Organic 0·0366%

Here again 89% of the Phosphorus is present in organic form. Therefore, the action of Pepsin on the artifical Nuclein is a prolonged though slight solvent one, no Nucleic Acid being split off from the Nuclein and practically no Phosphorus in the Tri -basic form. The previous researches on the action of Pepsin stated that it had no effect upon the Nucleins. This is only partially true with regard to the artificial one, namely in so far that the Phosphorus is not split off from the Nuclein, but the latter is merely slowly dissolved as such. (Albumin Nuclein).

Experiments were carried out to discover the action of Hydro-Chloric Acid alone on the Syntonin Nuclein, but practically no effect except a very slight solvent one was to be observed. No Nucleic Acid was present in the filtrate and only traces of Phosphorus in the tribasic form. (For nearer details see tables - 1+2).

Action of Trypsin on the Syntonin Nuclein.

Action of Trypsin on this Nuclein.

The next matter to settle was the action of the Pancreatic juice on the artificial Nuclein. The resistance of the Nucleins to Pepsin has been emphasised by all investigators, while the action of Trypsin on the same has been a matter of controversy. In order to decide this point, I have carried out a series of investigations. Before I give my own results, allow me to state shortly the work of previous investigators. Bókay was the first to emphasise the resistance which Nucleins opposed to the action of Pepsin, at the same time he remarked that Trypsin was also without effect on the Nucleins. Later, Popoff denied the latter statement, saying that the Thymus Nucleins were gradually split up by the action of the Tryptic ferment. As, however, he carried out his investigations on the gland substance and not on the Nucleins themselves, his results are not of the highest importance. Sebelien has, however, shown that the Para-Nuclein of Casein is rapidly split up by the Tryptic ferment. The Trypsin solution employed by me was prepared according to the method of Kühne

and Chittenden, for an account of the preparation see Zeit. für ~~p~~kysxxc~~k~~em. Biologie.* The Pancreatic juice contained no Nucleic Acid either free or loosely combined, but it is always very rich in organic Phosphorus. As I wished to estimate the amounts of Phosphorus present in both forms in the filtrate after Tryptic digestion, it was necessary for me to estimate the organic and tribasic Phosphorus in the original digestive solution.

Examples.

4·081 g. Nuclein (reckoned water-free) without being dried at 105° - ~~was~~ were digested for 11 hours with 220 c.c. of the Trypsin solution at 38°. The undigested remainder was washed with water containing a little Hydro-Chloric Acid, then with Alcohol and Ether and dried ~~at~~ to a constant weight at 105°.

A. The Syntonin Nuclein was examined ~~first~~s of all in the pure state without being digested. It then contained 3·49% Phosphorus.

B~~.~~ The same Syntonin Nuclein, after being digested ~~wikk~~ for 6 hours with 100 c.c. Pepsin solution at ~~103~~ at. 38°, con~~a~~tained 3·859% Phosphorus

C. The remainder, after Tryptic digestion, weighed 0·302 g. gave 0.011 g. $Mg_2P_2O_7$ corresponding to 0·033 g. P or 0·993% Phosphorus.

From these three analyses one sees in the first

*For Literature on all departments connected with the Nucleins + Paranucleins, see list at end of paper.

place that in A- detachable Proteid was present, in B. one sees the true Nuclein after being ~~a~~acted upon by the Pepsin, in C. the Nuclein has lost the large percentage of the Phosphorus. Unlike the action of Pepsin, the Trypsin rapidly and markedly splits off the Phosphorus from the true Nuclein. In order to decide whether the same action takes place after the Trypsin has only been in contact with the Nucline Nuclein for a short period, I carried out a series of experiments of which I shall give a characteristic one. 1 g. Syntonin Nuclein, prepared in the usual way, was digested for 6 hours with 60 c.c- of the Trypsin fluid. At the conclusion of this period the Nuclein, which originally contained approximately 4% Phosphorus, possessed only 1·469% Phosphorus. From this one clearly sees that the action of Trypsin gradually splits off the Phosphorus of the artificial Nuclein. In what form is the Phosphorus ~~vessel~~ present in the filtrate? The filtrates, after very ~~long~~ prolonged action of the Trypsin, reacted Acid, after a shorter periods Alkaline or neutral. When the fluid re-acted acid Carbonate of Soda was added in smal l quantity in order to allow the ferment to act. After the filtrates had been acidified with Acetic Acid, they possessed ~~approximately~~ the property of precipitating Albumins and Albumoses, but from these filtrates

Nucleic Acid could not be prepared in the usual way, i.e. by means of a hot concentrated solution of Barium Hydrate. If one renders the filtrate weakly alkaline with Baryta water (cold saturated solution) then adds Alcohol in excess, a ~~think~~ thick white precipitate falls rapidly to the bottom of the vessel. On this precipitate being divided finely up in water and acidified with Acetic Acid, then filtered and the filtrate added to a solution of Albumoses or Albumins, a slight precipitation occurs in some cases. It is extremely doubtful if this organic Phosphorus holding Acid be really Nucleic Acid, while from solutions containing the latter its preparation is by no means difficult. In order to find out in what form the Phosphorus in the filtrate after digestion I carried out the following analyses.

aX. In the Trypsin fluid, after being subjected to a temperature of 38° for 14 hours, 0·0409 g. P was present in the tribasic form. (i.e. after self digestion)

b. While 0·164% P comprised the total phosphates.

B. In the Trypsin fluid, after 14 hours digestion of the Syntonin Nuclein, the proportions of the different Phosphates were as follows:-

a. In Ortho form 0·0504

b. Total 0·2034%. i.e. 0·087 g. Phosphorus (Total) has been split off through the action of the Trypsin from the Nuclein, while only 0·0095 g. has been split off in the ortho form, in other words 89% of the total Phosphorus in the filtrates after digestion of the artificial Nuclein with ~~Kapsin~~ Trypsin is present in organic form. The Proteid precipitating body, which is split off from the artificial Nuclein, is not Metaphosphoric Acid because ~~if~~ one boiling boiled the water, neither ~~bineither~~ does the proportion of Ortho-phosphates increase nor is the ~~prkn~~ precipitating Proteid power lost, both of which would occur were the ~~A~~ body Metaphosphoric Acid. After longer action of the Trypsin, the whole of the Nuclein passes into solution. After tryptic digestion a Proteid precipitating body is always split off from the artificial Nuclein. The Barium salt of this Acid can in some cases be prepared, but owing to the small quantities in which it is present it was impossible to make the analyses of it. After very ~~porlonged~~ prolonged action of Trypsin, the Phosphorus in the tribasic form seems to ~~decrease~~ increase at the expense of the organic.

Action of Carbonate of Soda (0·25% solution).

on the Syntonin Nuclein.

Action of Na_2CO_3

Is the decomposition of the artificial Nuclein due to the ferment in the Pancreatic juice or to the alkali?

Analysis.

2·4416 g. Syntonin Nuclein (reckoned water free) prepared in the usual way, ~~was~~ were digested for 15 hours with 50 c.c. 0·25% Carbonate of Sadd solution. At the conclusion of this period, the greater portion of the Nuclein had passed into solution, the remainder was then washed with water containing Hydro-Chloric Acid, then with Alcohol and last of all with Ether. The white powder was then dried at 105° to constant weight The undigested ~~remained~~ remainder weighed 0·245 g. The Phosphorus was estimated by Webull's method and was found to ~~represent~~ amount to 0·653%. Another portion of the Syntonin Nuclein was digested with the same amount of Carbonate of Soda for 12 ~~hurs~~ hours and then was found to contain 1·998% Phosphorus. After the Syntonin Nuclein was digested for longer periods than 15 hours with the Carbonate of Soda, all passed into solution. From the Tables one remarks that the Pancreatic juice acts more rapidly than the

alkali, but both in the same direction. e.g. after 6 hours digestion with 60 c.c. Pancreatic juice, the undissolved remainder contains less Phosphorus than after 12 hours digestion ~~of~~ with the same quantity of Alkali upon ~~the~~ approximately the same amount of the artificial Nuclein. In the filtrates, after the action of the Alkali, asin the case with Tyrpsin, Trypsin the Phosphorus was present to much ~~a~~ * the larger extent in the organic form. The Proteid precipitating body is also split off from the Syntonin Nuclein, but no Nucleic Acid ~~may~~ can be obtained, according to the usual Barium method. If one adds a slight amount of Acetic Acid to the filtrate after digestion with the Pancreatic juice or with the ~~Alakli~~ Alkali alone, a very indistinct clouding appears which then rapidly fades away. From both filtrates one can prepare the Barium salt of the Proteid precipitating body. It is highly probable that Nucleic Acid is split off by the action of the Pancreatic juice and also by that of the Alkali, but owing to its affinity for Albumoses it rapidly combines with the latter and cannot be easily split off from the combination. The nature of the Albumose~~s~~ Nuclein I shall have occasion to speak of later. I may be allowed here, however, to state that the combination between Nucleic Acid and Albumose

is a very firm one . One may sum up conclusions as to the action of Trypsin and Sodium Carbonate in the following way . The action is a similar one in both cases, the only difference being that the ferment splits up the combination easier than the Alkali alone They both split off the Phosphorus from the Nuclein in all probability in the form of Nucleic Acid or in a nearly allied body and this combining with the Albumoses or Alkali-Albuminates forms firm combinations, while in addition probably a small amount of Nucleic Acid is split off in the free state and this combines with the alkali to form a salt. The fact that the undigested remainder contains so little Phosphorus agrees with the hypothesis that the latter is not merely transferred from original nuclein to the Albumose equivalent but partly in thericher Phosphorus-holding free acid. In all probability there are some Albumose Nucleins which act in the same way as the free acid viz. with regard to the Proteid-precipitating power.I have tried the effect of Deutero-Albumose Nuclein on different Albumoses and Syntonin , but without noting any precipitation.

Syntheses of Albumose-Nucleins with an account of their nature and behaviour towards the digestive ferments.

Syntheses of the Albumose-Nucleins.

As previously mentioned, Nucleic Acid precipitates Albumoses as well as Albumins and Globulins out of their acid solutions. As we know nothing with regard to the natural Albumose-Nucleins, we cannot form a comparison between them and those artificially formed. Still for the very reason that we know so little about those bodies as existing in the animal organism, the importance of obtaining aknowledge of the artificially formed ones becomes more requisite. I prepared the different Albumoses from the preparation called Witte's Peptone, which consists almost entirely of Albumoses with only a small quantity of Peptone. In the first place I prepared Deutero-Albumose in order to investigate the nature of the combination between Nucleic Acid and this, the most soluble of the Albumoses The method I employed for the preparation was that originally described by Kühne and modified by Neumeister. As large quantities of the original preparation are required in order to obtain even small quantities of the pure Deutero-Albumose, owing to loss during dialysis and other reasons which I shall mention later, I was able to make only a few analyses, sufficient how-

Deutero-Albumose Nuclein.

ever to give one a general idea of the nature of these bodies. The method of preparation was as follows - 1.6495 grammes Nucleic Acid were dissolved in 150 c.c. boiling water, the mixture was then allowed to cool, Into this a concentrated solution of Albumose, (Deutero) to which a few drops Acetic Acid had been added, was slowly poured. The precipitate which appeared was of a fine white cloudy character and only settled very slowly. The upper fluid, which always appeared cloudy, was then siphoned off and centrifugalised. In this way one was able to obtain afair quantity of the precipitate which was purified by washing with distilled water weakly acidified with dilute Acetic Acid, then with distilled water and finally with Alcohol and Ether. The substance obtained in this way was in the form of a white powder which reacted per se acid and was fairly easily soluble in dilute acid solutions after prolonged contact with the same. One portion of the Albumose-Nuclein was then dried at 105° to constant weight and the Phosphorus estimated in the usual way. This first portion weighed 0.8543 and contained 6.32% Phosphorus.

The second portion (without being previously heated at 105°) was digested with Pepsinum Activum -Marquart's- and 100 c.c. 0.25% HCl for 7 hours at 38°. The Nuclein after di-

-gestion weighed 0·2607 gramme, the larger portion having thus gone into solution .This undissolved body contained 6·29% Phosphorus. Therefore although a large proportion of the original Albumose-Nuclein has been dissolved by the Pepsin only a small amount of the Phosphorus has been split off.

The Nuclein before and after digestion contains practically the same amount of Phosphorus.

The smaller molecule of the Albumose - Nuclein contains therefore, as one would expect, a higher Phosphorus percentage than the larger molecule of the Syntonin-Nuclein.

Analysis2

1·664 grammesNucleicAcid were dissolved in 150 c.c. boiling water and after cooling 2·753 grammes Deutero-Albumose dissolved in 200 c.c. water(acidulated with Acetic Acid) wereadded . A fine white precipitate fell slowly tothebottom of the vessel, leaving a white opalescent fluid above from which more precipitate was obtained by centrifugalising. The precipitate so obtained possed the characters of the Albumose-Nuclein previously described . In one portion the Phosphorus was estimated(naturally after the usual purification). It contained 5·04% . The other portion was digested for 9 hours with Pepsin ,but it was so rapidly dissolv-

ed that it was impossible to carry out an analysis of the undissolved remainder.

Although these combinations between Nucleic Acid and Deutero-Albumose showed themselves to be fairly easily dissolved by the Pepsin, still, on analysis of the undigested remainder, it was found not to have lost much of its original Phosphorus, if it had lost any at all.

As we shall see later on, the Phosphorus percentage of the original Albumose-Nuclein, before being subjected to peptic digestion, depends largely on whether there be an excess of Albumoses or Nucleic Acid present. The interesting point is this that this Albumose-Nuclein shares at least partially the property of the simple Albumose in being soluble in weak acid solutions and especially in Pepsin Hydrochloric Acid.

Combinations between Protalbumose and Nucleic Acid

Protalbumose Nuclein.

Nucleic acid precipitates Protalbumose out of its solution in an extremely fine powder which tends to clump together into a gelatinouslike mass at the bottom of the vessel and which is extremely difficult to obtain free from excess of Albumose as it is so easily soluble in weak acid solutions. The cloudy opaque fluid was centrifugalised

but this did not succeed in procuring a better preparation, so that although I worked with fairly large quantities of Protalbumose it was found to be impossible to get sufficient of the Nuclein (?) for analytical purposes. As the isolation of the individual Albumoses entailed so much trouble and loss of material, I turned my attention to the nature of the bodies which Nucleic Acid precipitates out of a solution of a mixture of Albumoses & Peptones as in Witte's preparation. I employed large quantities of the Albumose and the Nucleic Acid as I wished to investigate also the fixity of the combination formed.

Combination between Nucleic Acid and the joint Albumoses & Peptones.

8.858 grammes Nucleic Acid were dissolved in 800cc. distilled water (at boiling point), the solution was allowed to cool, then filtered and to the filtrate a solution of Witte's Peptone (of the strength of 20 grammes in 400c.c. water weakly acidified with Acetic Acid) was added. A precipitate fell slowly to bottom of the vessel, but it the supernatant fluid remained very cloudy. The former was separated from the latter and purified in the usual way by ~~the~~ washing with distilled water, Alcohol and Ether and then dried to constant weight at 105° C. In this the percentage of Phosphorus was estimated and found to amount to 2.68%. This precipitate probably contained an excess of Albumose, although the supernatant fluid from which it was removed still possessed the property of precipitating Albumins.

In order to see whether this supernatant fluid could fix more Albumose, an excess of Witte's Peptone (in solution) was added and the second precipitate purified in the usual way. It contained 1.32% P. It appears thus as if Nucleic Acid precipitated Albumoses in different proportions out of a weak or strong solution of the latter. Perhaps also Nucleic Acid attaches itself more readily to some of the Albumoses in Witte's Peptone than to others. After these combinations between Nucleic Acid and Albumoses had been digested for some time with Pepsin the large proportion passed into solution and what remained was not sufficient for an analysis. In all cases also the undigested remainders were of a slimy nature difficult to purify. For these reasons, it was advisable to try and find out some other method for the preparation of the Albumose Nucleins. The supernatant fluid appeared always rich in Albumose and seemed also to contain more or less Nucleic Acid in solution which seemed to have lost its power of precipitating the excess of Albumoses present, or perhaps the precipitating agents present were really Albumose Nucleins and not the free acid itself. In order to find out if the bodies suspended in the cloudy supernatant fluid, after removing by centrifugalising as much of the obtainable precipitate as possible, were Albumose-Nucleins I pursued the following plan. The solution was placed in a beaker on the water bath and when almost at boiling point, Ammonium Sulphate was added to saturation

saturation point. The precipitate which formed was removed and dissolved in as little Baryta water as possible, After removal of the Barium Sulphate by filtration, the solution was precipitated by Methyl Alcohol (which does not precipitate Barium Hydrate)
This precipitate was washed with water, Alcohol and Ether and dried to constant weight at 105° C and then analysed. It was found to contain only traces of Phosphorus. That is to say either no Albumose Nucleins were present in the solution or if they were, they could not be prepared by this method.

I shall now give an example in which the above method was employed. In the following example a larger quantity of Nucleic Acid was employed to precipitate the Albumoses than formerly. 9 grammes Witte's Peptone were dissolved in 200 cc. water acidified with a few drops of Acetic Acid . Then 8grammes of Nucleic Acid were dissolved in 800cc. water in the usual way and the solution was added slowly to the Albumose one, a precipitate settled slowly at the bottom of the vessel, while the fluid above remained extremely opaque. Both precipitate and fluid were further treated.

A. The precipitate was washed with distilled water weakly acidified with Acetic Acid and then with Alcohol and Ether and last of all dried to constant weight at 105° C and the Phosphorus in one portion estimated in the usual way. (Weibull)

It contained 3.535%P. Another portion of the precipitate was digested for 6 hours at 38° with 100cc. Pepsin Hydrochloric Acid. The undigested remainder contained 5.4216% P.

B. The cloudy supernatant fluid was next investigated. It was saturated with Ammonium Sulphate in the warmth and the precipitate dissolved in as little Baryta water as possible. After the removal of all the Barium Sulphate by filtration, the filtrate was precipitated with Methyl Alcohol and the Barium salt investigated.

It was found however to contain no Phosphorus.

The Nature of the Combination between NUCLEIC Acid and the ALBUMOSES.

12.893 grammes Albumose Nuclein (prepared from about 30g. Witte's Peptone & 10 grammes Nucleic Acid) were dissolved in 200cc. 1% Ammonia. To this Fluid 180cc. of hot concentrated Barium Hydrate solution (60 grammes Barium Hydrate in 180cc. water) were added.

The precipitate which slowly fell was finely mixed up with water, acidified with Acetic Acid and then, after being made up to about 300cc., kept boiling in the Paraffin bath for about 2 hours. The fluid was filtered hot and then allowed to cool. The precipitate was boiled in the weak acid solution for two more periods each of two hours duration, and again filtered. The filtrates after standing overnight were poured into a sufficiency of Alcohol to which conc. HCl was added in the proportion

of 15cc. to the litre.

In this way no Nucleic Acid could be obtained, or, in other words, the combination between Nucleic Acid and Albumoses is a firm one.

A comparison between
The Natural and Artificial Nucleins

We have in the preceeding pages noticed some of the characteristics of the artificial Syntonin-and Albumose-Nucleins with regard to Phosphorus percentage, behaviour towards Pepsin, fixity of combination, action of Trypsin and Alkalies etc.
It remains to compare the natural Nucleins with the investigated artificial ones under the various conditions mentioned above. I have examined the Nucleins obtainable from the Thymus Gland, the Nuclei of the red blood corpuscles of the Goose, Hen and Duck and from the Pancreas.

Leuko-Nuclein.

In the first place, the Leukonuclein of the Thymus Gland was examined. It was obtained by precipitating with Acetic Acid out of the watery extract of the true gland tissue. The precipitate was dissolved in Ammonia holding water and reprecipitated with Acetic Acid. This precipitation + re-solution was repeated four times.

Method of Preparation.

The body obtained in this way is Nucleo-Histon, a combination between Leukonuclein and Histon, the latter being a body with some of the propertiesof an Albumose.
In order to obtain the Leukonuclein from the Nucleo-Histon, it is only necessary to extract the latter with

a weak Hydrochloric Acid solution which removes the Histon and leaves the Nuclein. The Histon can be precipitated out of the acid solution by means of Ammonia, this being a characteristic property possessed by the Histon as distinct from allother known Albumoses.

The Nuclein obtained in this way was washed with distilled water containing HCl until Histon-free, then with distilled water until Chlorine-free and lastly with Alcohol and Ether. A portion of the fine white powder obtained in this way was then dried at 105° to constant weight and the Phosphorus estimated.

The Leukonuclein, as thus prepared, was found to contain 4.416% Phosphorus.

Action of Pepsin on the Leukonuclein.

Action of Pepsin on Same

4.128 grammes Leukonuclein (reckoned water-free) were digested 10 hours with 100cc. Pepsin solution at 38°, when about onethird was found to have passed into solution. The undigested remainder was purified in the usual way, washed with Alcohol and Ether and dried at 105° to constant weight, incinerated by Kjeldahl's method and Phosphorus estimated. It was found to contain 4.325% Phosphorus i.e. a loss of 0.091% P. from the action of Pepsin.

In what form is the Phosphorus present in the filtrate after Peptic digestion? In the first place, the filtrate precipitates neither Syntonin nor Albumoses out of their acid solutions, and no Nucleic acid could be obtained from the dissolved digested products.

Secondly, 91.322% of the total Phosphorus present in the filtrate was in organic combination, and only 8.678% in tribasic form. Thirdly, the total amount of Phosphorus in the filtrate derived from the Leukonuclein being 0.0484grammes while only 0.0037grammes P. is split off from the same, the difference between the two consists of Phosphorus derived from the Nuclein without altering the percentage of the same.

In other words:- 4.128g Nuclein after 10 hours peptic digestion loses 0.0037g Phosphorus and in the Pepsin filtrate after digestion 0.0484g P is present, this leaving 0.0447g P either in the form of Nuclein dissolved simply as such, or as Albumose Nuclein.

Of the total Phosphorus of the original Nuclein, only 7.65% is split off from the same and this appears to be completely in the tribasic form. Thus here as in the case of the artificialSyntonin-Nuclein, the natural one shows great resistance to Pepsin when the latter is looked upon as a splitting up agent. The Pepsin however slowly dissolves the Nuclein. No Nucleic Acid is split off, but the P. is present almost entirely in the filtrate in organic form. The next question as to the fixity of the combinationbetween the components of the Nuclein of the Thymus has been answered by others-(Kosseland Lilienfeld). From an Alkaline solution of the Leukonuclein, the Nucleic Acid is split off by the action of hot concentrated Baryta solution. That is to say the

combination between the Nucleic Acid and the Proteid is a loose one and therefore distinct from that existing in the Syntonin-and Albumose Nucleins.

Action of Pancreatic Juice on the Leukonuclein.

Action of Trypsin the same.

This has not been previously investigated and as we have seen the comparative resistance which this Nuclein offers to Pepsin, it was important to find out whether the same holds good with Trypsin.

First of all, the Leukonuclein was subjected to the action of Pepsin for a short time and the undigested remainder divided into two portions. The one was further purified by washing with distilled water, Alcohol, and Ether, then dried to constant weight at 105° and the Phosphorus estimated. It contained 4·32%.

Another portion of the peptic digested Nucleinwas (after washing with distilled water until free from acid) subjected to the action of 60 c.c. Pancreatic juice for 4 hours at 38°. Rather more than the half had passed into solution. The undigested remainder was washed with weak HCl-holding water, distilled water, Alcohol, and Ether and lastly (with care) dried at 105° to constant weight. The Phosphorus percentage, estimated in the usual way, was found to amount to 1838 %. The filtrate from the Nuclein digested with Pancreatic juice, reacted weakly alkaline at the close of digestion, and precipitated on the addition of Acetic Acid, Albumoses and Syntonin out of their solutions.

1·838% P

By the Baryta treatment, no Nucleic Acid could be obtain-ed. Only 8,373%P. was present in the tribasic form.

In what form then is the P. present in the filtrate? The Nuclein before digestion contained 0,0537g. P.: After Pancreatic digestion 0.0309g. of this was split off and passed in soluble form into the filtrate, i.e. 57.55% of the original Phosphorus in the Nuclein .

As we have seen above only 8.373% is present in the filtrate in the tribasic form, therefore even in the case of the Phosphorus split off from the Nuclein, the large percentage exists in organic combination.

Nucleic Acid does not appear to be free in the filtrate, not even in the form of a salt. Probably even if the Nucleic Acid were as such present in a mixture of Albumoses and Peptones it would unite with the latter at once to form fixed combinations.

That the fluid posesses the power of precipitating Albumoses &c after it has been acidified with Acetic Acid may be due to many other causes.- First the pres-ence of an acid nearly allied to Nucleic Acid and shar-ing with it this property, but not that of fixity of combination with Albumoses or Peptones would account for it. Or a very small amount of Nucleic Acid may really be present sufficient to precipitate Albumoses; but not in sufficient quantity to allow one to obtain it by the Baryta method. Or again Nucleic Acid may form a combination with some Albumoses which possesses the

proteid-precipitating power of the free acid. We must not at once jump to the conclusion that because practically all the Phosphorus in the filtrate is present in organic form and that because at the same time a proteid precipitating body is present, that therefore it must be Nucleic Acid. That the proteid -precipitating power is not due to Metaphosphoric Acid is shown by the fact that boiling does not diminish this property. If Metaphosphoric Acid were present, boiling in the presence of acids would transform it into Orthophosphoric Acid and this, of course, would not be able to precipitate Albumoses out of their solutions. Paranucleic Acid shares the proteid precipitating power with Nucleic Acids and as we shall see later, its combinations with Proteids are much easier soluble in weak HCl than those of Nucleic Acid. The proteid-precipitating body split off from the Leukonuclein by the action of Trypsin and Carbonate of Soda forms combinations with Syntonin and Albumoses which are also easily soluble in weak Hydrochloric Acid, in fact even more so than the artificial Paranucleins. As I shall show later, the proteid-precipitating body present in the yolk of the egg precipitates Syntonin out of its solutions, and that the bodies so obtained are also very easily soluble in 0·25% HCl.

Action of Na_2CO_3 a same.

Action of Carbonate of Soda on the Leukonuclein.

Is the marked decomposing action of the Pancreatic juice on this Nuclein due to the Trypsin or to the alkali or both combined?

1•1402 grammes Leukonucleøin were digested with 60cc 0•25% Carbonate of Soda for 4 hours at 38°, that is to say under the same conditions as in the case of the Pancreatic juice. Scarcely the half passed into solution (not quite so much as after the action of the Pancreatic juice). The undissolved remainder was, after the usual purification and drying, incinerated by Kjeldahl's method and Phosphorus estimated after WEIBULL

It was found to contain 2•503% Phosphorus.

In what form is the Phosphorus present in the filtrate? Firstly, only traces are present in the ortho- form, viz., 4•737%.

The filtrate reacts slightly alkaline, although there is always a loss in the ~~basicity~~ alkalinity. On acidifying, the filtrate precipitates Albumoses and Syntonin, the bodies so formed being easily, however, soluble in excess of either Acetic Acid or Hydrochloric Acid. By the Baryta method neither Nucleic Acid nor Paranucleic Acid can be obtained.

To sum up, ~~with~~ Carbonate of Soda of the same strength

as that which occurs inthe Pancreatic juice, under the same conditions, isx able to split up the Leukonclein in the same way though to a slighter extent than the Trypsin. The Phosphorus ofthe original Nuclein is split of in a soluble organic combination which ~~possess~~ possesses ~~thes~~ the proteid-precipitating power characteristic of the Nucleic and Paranucleic Acids. That this bodyis not Monometaphosphoric Acid follows from the fact that boil in acid solution does not alter this property.

Action of Hydrochloric Acid ont the Leukonuclein.

The action of the acid alone at body temperature and when used ofthe same strength (0 25%) as in the case of the Pepsin was found to be practically only that of a slight solvent. The Nuclein ~~did not lose~~ was only ~~lost~~ slightly poorer in Phosphorus percentage, while in the filtrate neither Nucleic Acid nor tri-basic Phosphorus were to be found and only traces of Phosphorus in organic combination.

Nuclein of red blood corpuscles of Birds.

The Nuclein of the red blood corpuscles of the bird.

The red corpuscles, which I examined first of all, were obtained from a mixture of the blood of the goose and hen. The red corpuscles were treated in the following way in order to obtain the Nucleins fromthem.

To every 100 cc of defribinated blood, 90cc of a cold saturated solution of Natrium Sulphate (Na_2SO_4) and ~~840~~ 810cc. 810cc of distilled water were added. After thorough mixture, the blood (diluted in this way) was poured into broad shallow dishes with flat bottoms and then left in a cool place for two days. At the end of this period the corpuscles had settled in a layer at the bottom of the vessel. The supernatant fluid was siphoned off and then a very small quantity of water which had been warmed at 40° was poured into the vessel in order to dissolve the corpuscles. The pink fluid so-obtained was poured into a separating funneland a small quantity of Ether was added to assist in solution of the Haemoglobin. The vessel, after being gently shaken, was then allowed to stand until the Nuclei of the red corpuscles had separated out as a welldefined layer between the lower watery layer containing the blood pigments and the upper ethereal layer. When the middle layer became well defined, the lower fluid was drawn off and then the upper and finally the intermediate one was removed

by means of a spoon-like apparatus. This nuclear mass was then placed in a vessel containing a very large quantity of water (2-3 litres water for every litre blood originally taken) to which a little Ether had been added and the fluid was then thoroughly well mixed up. The nuclei fell to the bottom of the vessel. The upper fluid ~~is~~ was then siphoned off and the washing process repeated until the ethereal water ~~can~~ could remove no more of the colouring matter. The nuclei (which ~~are~~ were now almost colourless) were then allowed to stand in a 0·25% solution of Hydrochloric Acid in order to dissolve the Histon. The solution was left over-night and then the upper fluid siphoned off. The Nuclein obtained in this way always contains more or less Lecithin and Haematin. In order to remove these, the impure Nuclein was extracted for about 12 hours at 40° with Alcohol containing a few drops of Hydrochloric Acid, then with Ether and finally dried in exsiccator. The Nuclein obtained in this way is in the form of a greyish coloured powder and is free from Histon and Lecithin etc. In one portion the Phosphorus was estimated in the usual way and was found to amount to 5·914%. Another portion was digested with Pepsin for 7½ hours and the Phosphorus in the undissolved remainder (after the latter had been purified and dried) was estimated by Weibull's method. It contained 5·809% Phosphorus i.e.

practically the same as before digestion. The pepsin filtrate precipitated neither Syntonin nor Albumoses and contained no Nucleic Acid. In the pepsin solution originally 0·0061%P was present in the trib asic , 0·0011%P in the organic form. In the Pepsin filtrate after digestion of the Nuclein 0·0106%P was present in the tribasic form and 0·0071% in the organic that is to say of the total Phosphorus present in the soluble form in the filtrate derived from the Nuclein by the action of Pepsin 42% is in ortho- combination and the rest in organic form.

Before I refer to the action of Trypsin on this Nuclein, I shall, for the sake of continuity, give my analyses of the Nuclein of the red blood corpuscles of the goose , when prepared in the same way and subjected to the action of Pepsin etc.

I used large quantities of blood (2-4 litres) in order to obtain sufficient material to investigate the action of the digestive ferments on the same, and also to find out the nature of the combination in the Nuclein and its behaviour under the action of alkalies. I found great difficulty in obtaining this Nuclein Haematin-free, and in trying to do this I lost a large quantity owing to the long extraction with acid alcohol (loss through siphoning, filtration etc.). In addition my analyses of the Phosphorus percentages is in all pro-

bability rather low because of the slight decomposing action of the warm acid alcohol. After the Nuclein had been prepared in the way previously described and purified in the routine way, the Phosphorus was estimated in one portion (0·4437gm) and wasfound to amount to 4·248%

Action of Pepsin on this Nuclein.

1·6147gm (reckoned water-free) were digested with 50cc Pepsin-HCl for 8½ hours at 38°. THE weight after digestion was 0·864gm. This was incinerated, but, unfortunately, owing to an accident, was lost. The filtrate precipitated neither Syntonin nor Albumoses in Acetic Acid solution. It contained (the Phosphorus inthe tribasic and in the organic forms in the original Pepsin being naturally subtracted) 0·0090%P in the ortho-, 0·0483%P in the organic form, or, in other words, 84%organic_ 15·71%inorganicP ·

Action of Trypsin on the Nucleins of the red corpuscles of the birds' blood.

The Nucleins were prepared in the way above described and subjected to the action of Trypsin for varying periods. This was especially done in order to find out the form in which the Phosphorus was split off from the Nuclein. Trypsin and the alkalies always do split up the ~~alk~~ Nucleins, The only way of obtaining a real

knowledge of the specific action of the digestive ferments on different Nucleins ~~is~~ consists in the combined examination of residue and filtrate. In all cases the larger percentage of Phosphorus is split off in the organic form; but in some cases the amount of inorganic is almost as great. Then often there seems to be a variation in the nature of the organic Phosphorus holding body split off. In some it is split off in such marked quantity as to lend to the filtrate most distinct proteid precipitating properties, in others this power has almost been completely lost. In some the combination formed between these precipitating bodies and different Albumins and Globulins and albumoses are of a very soluble nature, in others not at all so.

~~Action of Trypsin on the above-mentioned Nucleins with regard to the form in which the P is split off.~~

After the Nuclein had been acted upon by Pepsin for 7 hours, the undissolved remainder was purified in the usual way and after having been dried in the exsiccator was subjected to the further action of Trypsin for 7½ hours. In the case of the Nuclein of the duck, s red blood corpuscles, the solution, after this period was an almost complete one.

The filtrate precipitated Syntonin and Albumoses out of their acid solutions to a very slight degree merely. In what form then was the Phosphorus present in

the filtrate ? In the first place, 47·223% Phosphorus was present in the filtrate in the tribasic state, the rest being in organic combination. The proportions here are quite different from those existing after tryptic digestion of other Nucleins. The filtrate was precipitated with a hot concentrated solution of Ba. Hydrate and the precipitate which fell, after being finely divided up, was slightly acidified with Acetic Acid and boiled on the paraffin bath for two hours, then filtered and the filtrate tested for Nucleic Acid. The filtrate so obtained could precipitate neither Syntonin nor Albumoses and gave only a slight cloudiness on being poured into Alcohol-HCl. As it was possible that although Baryta in the warmth did not precipitate Nucleic Acid out of its solution or combination from the fact that the Barium salt might be soluble in excess of Barium Hydrate, the filtrate after precipitation with the Baryta was treated with great excess of Alcohol. The precipitate obtained in this way, was treated in the same way as the former one; but no Nucleic Acid was found. It seems almost certain that Trypsin does not split off Nucleic Acid Acid or a body similar to that acid from the Nucleins of the red blood corpuscles of the hen, duck and goose (as we shall see immediately).
the Nuclein of the red blood corpuscles of the goose

was examined in the same way.

0·9827gm. Nuclein was digested for 7 hours with 50 c.c. Pancreatic juice. Practically all passed into solution. The filtrate, which measured 40c.c. reacted alkaline after digestion. The filtrate was divided into three portions, each of which was separately examined as follows-

(a) In one portion, the tribasic P was estimated

(b) In the next, the total P was reckoned.

(c) In the last, the Baryta method was employed in order to discover if Nucleic Acid were present.

This was done in exactly the same way as described for the Nuclein of the hen's blood. No nucleic acid was obtained. The Tryptic filtrate after digestion did not precipitate Syntonin nor Albumoses out of their acid solutions, in this respect showing a marked resemblance to the Nucleins of the Goose and Hen. With regard to the proportion of tribasic to organic Phosphorus in the digestive products after the action of Trypsin, there is a marked difference here compared to that noticed in the case of the Nuclein of the Duck's corpuscles. Only 6%P. was split off in the inorganic form, the rest being organically bound. This may have been due to a weaker pancreatic infusion.

The points of importance are, in the first place, that in no case is a proteid-precipitating body

split off in appreciable quantity from the Nucleins of the red blood corpuscles of the Goose, Duck, or Hen, through the action of Trypsin. Secondly, that Pepsin ~~spl~~ splits off a much larger proportion in the tribasic form than in the case of the other Nucleins.

In order to find out whether the combination between acid and Albumin in the ~~Nuclein~~ molecule of the Nuclein of the bird's blood be a firm or a loose one, I dissolved a quantity of the Nuclein of the Duck's blood in 1% Ammonia and treated the solution with a hot concentrated solution of Barium Hydrate, and then boiled the precipitate so obtained in water acidified with acetic acid on paraffin bath for 1½-2 hours. The solution was then filtered and the filtrate tested for Nucleic Acid. None was obtained in this way, so one may conclude that the combination is a firm one.

See note a page 110 as to the presence of a carbohydrate molecule in this Nuclein.

Nucleo-Proteid of the Pancreas.

The Nucleo-Proteid of the Pancreas.

This Nucleo-Proteid has been quite recently investigated with regard to its decomposition products by Hammarsten. He termed it a Nucleo-Proteid because out of its solution in acid on digestion with Pepsin a true Nuclein separated out ~~fell~~. That the body was a true Nuclein followed from the fact that on decomposition with acids in the heat, it furnished Guanin. In addition he obtained in the same way CuO reducing bodies which in

all probability were Pentoses. Unfortunately the author has not been able to finish the work completely.

As Hammarsten did not examine the nature of the bodies split off by the action of Pepsin nor did he investigate the effect of Trypsin on the same, Ihave tried to fill up these blanks.

The Nuclein was prepared inthe way described by Hammarsten. The finely minced-up gland substance of the Pancreas was boiled for a long period with water and the watery extract after being filtered was precipitated with about 5pro Mille acetic acid. The body obtained in this way was dissolved in weak Ammonia and the solution in its turn precipitated with AceticAcid. I did not carry out this process of precipitation and solution so long as Hammarsten, as I had not a large quantity of material to work with. I may say at this place that on reading H's paper one obtains no idea of the exceedingly small amount of Nuclein to be obtained even from a large amount of Pancreas. When I say that ~~although~~ I used more than ten kilogrammes of glands to obtain about 20gm. of the Nuclein, one receives an impression of the difficulties and expenses incurred in carrying out a long series of experiments.

~~This~~ body, after washing with Alcohol and Ether, was dried to constant weight at 105° and the Phosphorus estimated. It contained 2.796% P. This is a much lower percentage than that found by Hammarsten, probably the difference being due to the larger amount of Albumin

attached to the Nuclein molecule in my preparation.

. . . . . .

Action of Pepsin on this Nuclein.

Action of Pepsin on Same.

As I could not dissolve the Nucleo-Proteid in 0·25% HCl after extraction with Alcohol and Ether, I simply took a weighed quantity and digested it with ~~Alcohol~~ Pepsin.

1·2246 gm. were digested 6 hours with 50 cc Pepsin at 38°. The undissolved remainder, afterpurification etc, was found to contain 3·196% Phosphorus. 3·196% P.

In what form is the P. present in the filtrate?

3·18

Only 3·18% P. is present in the ortho- form. The filtrate showed slight cloudiness on the addition of a few drops of Acetic Acid, soluble in HCl. On neutralisation, no precipitation occurred. On the addition of Albumoses to the filtrate after it had been acidified with AceticAcid, a marked precipitation occured which was easily soluble in weak Hydrochloric Acid not in weak Acetic Acid.

This precipitation is noteworthy as showing that Pepsin is capable of splitting off a proteid precipitating body from the Pancreas Nuclein.

One part of the filtrate was made alkaline with a cold saturated solution of Barium Hydrate and then Alcohol was added in excess. The precipitate so obtained on being dissolved in water did not precipitate Albumoses. Of the total P. of the Nucleo-proteid 20·65% P. passed into solution after 6½ hours

peptic digestion, and this is present almost entirely in organic combination.

.....

Action of Trypsin.

The action of the Pancreatic juice on this Nuclein.

1·1876 gm. (reckoned water free) Pancreas Nuclein were digested with 70 c.c. Pancreatic infusion for 7 hours at a temperature of 38°. Only 0·075 gm. remained undissolved, and this was found to contain 1·12% Phosphorus.

In what form is the P. present in the filtrate?

(a). On Albumose solution being added to the filtrate after the latter has been acidified with Acetic Acid, a precipitation at once occurs which is easily soluble in dilute HCl but not in weak Acetic Acid.

(b). The filtrate was in the first place acidified slightly with acetic acid, then made weakly alkaline with a cold saturated solution of Barium Hydrate , filtered and the precipitate obtained in this way dissolved in water. This watery solution pre cipitates Albumoses out of their acid solutions. This shows that this proteid -precipitating body shares with Paranucleic Acid (and at least one Nucleic Acid) the property of forming soluble salts with Barium. This also serves to distinguish all these bodies from Metaphosphoric Acid (I speak only of Monometaphosphoric Acid not of the polymers eg. Trimeta-

phosphoric acid. Unfortunately the quantity of the precipitate was so small that the Ba. and P. could not be estimated.

(c). In the filtrate, as usual, by far the larger quantity of the P. was present in the organic form, but the inorganic was proportionately greater in amount than after peptic digestion viz. 76·47%P. in organic form. Of the total P. of the original Nuclein about 53% passed into solution after 7 hours pancreatic digestion. [In order to arrive at these results, the method employed was as follows- the P. in the Nuclein before digestion was estimated. In 1·1876 gm. ~~was~~ P was present 0·03094 gm., while the total P. in the filtrate obtained by digestion of the Nuclein amounted to 0·0166 (i.e. naturally with the P. of the original infusion subtracted), and the inorganic to 0 0056%P.]

Here as in the case of all the Nucleins examined, by far the larger percentage of Phosphorus in the soluble digestion products is in organic form. This agrees with Salkowski's results on the Paranuclein of Casein against those of other investigators who found that peptic digestion of the above mentioned Paranuclein caused an increase of the ortho-Phosphoric acid in the digestion products. This is of extreme importance, - that the Phosphorus must be absorbed in an organic combination even after prolonged Peptic and Tryptic digestions.

A comparison between the

natural and artificial Paranucleins.

The differences between the so-called Para- or Pseudo Nucleins and the true Nucleins, I have already pointed out. Unfortunately our knowledge of the two classes is of such a limited nature (this especially appliesto the former) that it is impossible at present to do more than point out that there is a class of bodies containing Phosphorus organically bound which is intimately connected with the life of the cell and which distinguishes itself from the class of true Nucleins in not furnishing Nuclein bases on decomposition. That such bodies as Walther's Ichthyulin , the Paranuclein of Casein, and the Paranuclein of Ovovitellin of the yolk of the hen's egg, all possess this negative character in common and yet differ in most important other points, shows that, under the term Paranuclein, bodies of entirely different constitution are included. Just let me recapitulate some of these important differences.

Walther's Ichthyulin furnishes on decomposition with acids a CuO-reducing body, while neither of the other two do. Secondly, from the Paranuclein (so-called) of the Ovovitellin of the yolk of the hen's egg, a body is split off by the action of alkalies which is quite distinct from both Nucleic and Paranucleic Acids (by the latter I mean the acid obtained from the Nucleic

Acid of the Thymus gland). I shall come back to this body later .

With regard to the Paranuclein of Casein , we know extremely little. Clara Willdenow, Salkowski and Hahn have mentioned that, after Peptic digestion, a body is split off which can precipitate Proteids out of their acid solutions. This body is precipitable by Acetic acid and can be in this way removed from its solutions Salkowski pointed out that, on boiling with a solution of Barium Hydrate, all the Phosphorus of this body was slowly transformed into the tribasic form, though with much greater difficulty than Metaphosphoric Acid On Peptic digestion, the P. passed slowly into solution inthe form of the digestive products. By far the larger proportion of the P. of the original Casein is in the soluble form after digestion and only a comparatively small quantity remains in the Paranuclein.

The bodies which Zaccharias has termed Paranucleins have not yet been examined chemically and we must wait until those phosphorus-holding bodies which are so abundant in the seeds of different plants, have been made the subject of careful investigation.

Thanks to the investigations of Kossel, we are in possession of certain facts which enable us to get a much clearer idea of the nature of these exceedingly complicated bodies. When Nucleic Acid in a solution of

of boiling water has been kept on the water-bath for a short time (10-15 min.) at a temperature of about 90° some of the Nuclein bases are split off and also a small quantity of a rich N-holding base of the formula $C_{21}H_{30}N_{16}O_4 + 5H_2O$, and along with these a richer P-holding acid than the Nucleic acid. This latter body still possesses the proteid-precipitating property of the original acid; but is no longer precipitated by HCl. This acid has in all probability the formula $C_{16}H_{25}N_3O_{12}P_2$. NO Phosphorus is split off at the same time in the tribasic form. When the Nucleic Acid is acted upon by even weak acids in the warmth, the Paranucleic Acid is split up into a series of bodies not yet definitely characterised. The points of importance are that the proteid-precipitating power is early lost, the P. evidently passing into the ortho-form, and, secondly, that among the other decomposition products there are Formic Acid, Thymin, Ammonia, and, what is of extreme interest, Levulinic Acid. The latter acid ~~only~~ is obtainable only from Carbohydrates according to Tollens. One other important decomposition product is a body which possesses neither acid nor alkaline characters-Thymin-with the formula $C_5H_6N_2O_2$ (new formula). What still requires to be done is the working out of each step in the process of decomposition and this applies also to the different Pseudo-Nucleins.

Synthesis of the Paranucleins

The artificial Paranucleins.

Paranucleic Acid precipitates Albumins, Globulins and Albumoses out of their acetic acid solutions. These precipitates are easily soluble in alkalies, being also partially split up at the same time. They react acid and give the ordinary proteid reactions.

I have investigated them in the same way as the artificial Nucleins.

Method of preparation of the Paranucleic Acid. (acc. to Kossel).
(not yet published)

Method of Preparation of Paranucleic Acid.

A one percent. solution of Nucleic acid was prepared in the usual way and after being filtered was kept at a temperature of about 90° for 15 min. The solution was then tested for the presence of Nucleic acid with a few drops of HCl. If precipitation or even cloudiness occurred, the solutionwas kept a little longer on the waterbath and then again tested. On the disappearance of all signs of precipitation (absence of Nucleic Acid) on the addition of HCl, the heating was discontinued. The solution was in the next place tested for Phosphates with a little Baryta water. None should be present. The solution is now allowed to cool, and then a cold saturated solution of Barium Hydrate is added until the solution is faintly alkaline. About an equal quantity of Alcohol (equal to the total amount of fluid) is now added and the precipitate allowed to settle overnight. If the precipitate does not fall properly a little more Baryta should be added. The precipitate

is then to be dissolved in as little water as possible and the solution again precipitated with Alcohol. This process of solution is to be repeated thrice The Barium salt of the Paranucleic Acid is, when prepared in the way just described, in the form of a fine white powder. On being dissolved in a weak acetic acid solution, the Paranucleic Acid is set free and may then be used to precipitate ~~pt~~ Proteids out of their solutions. The Proteid solution which I employed was one of Syntonin in Acetic Acid (2%)

I shall now give the analyses:-

(1). 5 grammes Nucleic Acid were dissolved in 250cc boiling water and from this the Paranucleic acid was prepared in the way above described. A solution (1%) of Syntonin was added to that of the Paranucleic Acid in acetic acid. until no more precipitation occurs. A very thick precipitate falls slowly to the bottom of the vessel. This was washed thoroughly with distilled water and then extracted with Alcohol, and Ether . In one portion the P. was estimated in the usual way.

0·274gm. gave 0.027g $Mg_2P_2O_7$ = 2·755% Phosphorus (2·755%P.)

Another portion (1·4685gm. free H_2O-reckoned) was digested for 16 hours with 100cc Pepsin-HCl, then filtrated and both filtrate and undissolved remainder examined. The latter was, after purification, dried

are these Kossel and Yes?

No - they are my own. Kossel devised the method for preparing Paranucleic (or as he now terms it Thymic) Acid. He has neither prepared nor analysed the artificial Paranucleins

Diff.

Action of Pepsin on the artificial Syntonin Paranucleins.

to constant weight at 105, and the P. percentage estimated. The body before incineration weighed 0·133gm and contained 5·045%P.

0·133gm furnished 0·024gm $Mg_2P_2O_7$
= 5·045 % P.

The filtrate was examined in the following way:-
One portion was made weakly alkaline with a cold saturated solution of Barium Hydrate and then precipitated with Alcohol and the precipitate dissolved in water. This watery solution precipitated Albumoses to a marked degree out of their acid solutions. i.e. a Barium salt of Paranucleic acid.
In two other portions the total and tribasic Phosphorus were estimated. 92% was present in organic form. Of the total P. of the original Paranuclein, 90% passed into solution after 16 hours digestion with Pepsin.

Analysis (2).
The Paranuclein was prepared in the usual way and the P. estimated.

0·1923 gm. gave 0·0209 gm. $Mg_2P_2O_7$ = 0·0058 gm. P.
= 3·01%P.

A portion of this same Paranuclein was subjected to the action of Pepsin, but all passed into solution.

Analysis (3).
1·895 gm. of the Barium salt of Paranucleic Acid was dissolved in 100cc water, acidified with a few drops acetic acid and then a solution of Syntonin was added and the precipitate which wasformed was treated

in the usual way.

One portion was incinerated and the Phosphorus estimated . It was found to contain 2·8% P.

Action of Pepsin on this Paranuclein.

1·199 gm. (reckoned water-free) were digested with 60c.c. Pepsin HCl for 6 hours at 38°. Solution only occurred very slowly and at the end of digestion the undissolved remainder weighed 0·849gm. and contained 3·286%P.

The filtrate did not precipitate Syntonin out of its Acetic acid solution.

Analysis (4).

The Barium salt of Paranucleic Acid was prepared from about 3 gm. Nucleic Acid and an acetic acid solution of this was employed to precipitate Syntonin out of its AceticAcid solution. The Paranuclein so prepared was purified in the usual way and the P. in the same before and after digestion estimated. (Weibull's method)

0·6287gm. furnished 0·053gm. $Mg_2P_2O_7$

= 2·351% P.

Action of Pepsin on the same.

3·751 gm. (reckoned water-free) Syntonin-Paranuclein were digested for 18 hours at 38° with 150 c.c. Pepsin HCl. Almost the whole quantity passed into solution the undissolved remainder only weighing 0·238 gm.

On analysis this was found to contain 4·34%Phosphorus

The filtrate precipitated Syntonin out of its acetic

acid solutions. ~~TH=E~~ The filtrate was precipitated with a hot concentrated solution of Barium Hydrate. Neither the watery nor the acetic acid solution of this precipitate caused even cloudiness in a Syntonin solution . The same applies to the alcohol precipitate obtained from the filtrate after removal of the Barium precipitate. As neither the Barium precipitate nor the filtrate from the same possessed the proteid-precipitating property, the hot concentrated Barium Hydrate ~~is~~ must be the agent that removes this property . This is in all probability a transference of the Phosphorus from the Meta- into the Ortho- form.

Action of Trypsin on this Paranuclein

Action of Trypsin.

0·9696 gm. (reckoned water-free) Paranuclein was digested for 10 hours with 60 c.c. of a Pancreatic infusion at a temperature of 38°. Only a small quantity remained undissolved and this contained 1·584% Phosphorus

In the filtrate only 3·93% P. was in the tribasic form The filtrate reacted alkaline and on acidification with acetic acid precipitated Albumoses and Syntonin Both precipitates were soluble in Hydrochloric Acid.

Conclusions as to the nature of the artificial Syntonin-Paranuclein.

(1). Paranucleic Acid precipitates Syntonin and Albumoses out of their acetic acid solutions to quite as great an extent as Nucleic Acid.

(2). These precipitated bodies react acid, are easily soluble in alkalies and also in weak mineral acids after prolonged action and in stronger acids (mineral) after a shorttime.

(3). Paranucleic Acid combines with Syntonin to form not a true Paranuclein , but that body plus a large quantity of adherent proteid removable by the action of Pepsin cp. especially in this respect the percentages given in Analysis(1)

(4). The body formed first of all on the addition of Paranucleic Acid to a Syntonin solution contains a fairly constant Phosphorus percentage viz., from circa 2·4% - 3%

Gradually the Pepsin removed the excess of adherent proteid , so that , after 16 hours digestion , the percentage of Phosphorus rose to about 5%

After more prolonged action of the Pepsin, there is a loss of Phosphorus . Still the Paranuclein seems to be very resistant to the action of Pepsin, except in so far that it is slowly dissolved by it.

(5). Almost the entire Phosphorus in the digestive products is in organic combination, at least only an extremely small quantity is in the tribasic form. After prolonged action of the Pepsin, a proteid-precipitating body is split off from the Paranuclein which loses its characteristic property on being acted upon by a hot

concentrated solution of Barium Hydrate , similar to the transformation of a Meta- into an Ortho- Phosphoric Acid.

(6). Trypsin and, in all probability, the Na_2CO_3 also rapidly splits up the artificial Paranuclein, the Phosphorus passing over into the soluble digestive products almost entirely in organic form. The proteid-precipitating body split off by the action of the Trypsin is in all probability the same as that appearing after the action of Pepsin.

The natural Paranucleins.

The natural Paranucleins

Our knowledge of these bodies is of a very limited nature. I have already described almost all that is known of them at the present day. The Paranuclein which I have investigated is that described by Bunge as occurring in the Yolk of the hen's egg and termed by him Haematogen. It contains Iron and Phosphorus in organic combination.

There are at least two methods by which one can prepare this body. First of all that given by Bunge VIZ., solution of the yolks of eggs (whose colouring matter had been completely removed by extraction with Ether) in 0·25% HCl and then digestion of the mixture with Pepsin when a white precipitate separates out .

This body contains Iron and Phosphorus in organic com-

bination in addition to C, H, N, O, S . There is great difficulty in getting this body free from Lecithin. It is by no means unlikely that the Lecithin is really in combination with the Paranuclein (Hoppe-Seyler , Liebermann).

The second method is as follows:- The yolks of 30-50 eggs are extracted with Ether until the latter can extract no more colouring matter. The residue is then dissolved in 10% Sodium Chloride solution and this precipitated by adding excess of water . A white flocculent precipitate falls to the bottom of the vessel which is then in its turn redissolved in 10%NaCl This is to be repeated two or three times . The Ovovitellin purified in this way by reprecipitation is now dissolved in 0·25% HCl and digested with a Pepsin infusion about 36 hours. A white precipitate falls out though in a much smaller amount than after Bunge's method.

I have employed both methods and shall now give a short account of the same.

I shall in the first place speak of Bunge's method. If the method of preparation is carried out exactly as the author describes ,abody is obtained with a P. percentage approximately the same as that given by him viz., 0·5 % ;but the longer it is left in contact with even a weak alkali especially NaOH , the lower

does that percentage become until after repeated solution in NaOH and precipitation with AceticAcid, it becomes Phosphorus free. The form in which the P. is split off, I shall describe later. In all probability Alcohol to which a few drops of HCl have been added (especially if the action take place in the warmth) acts in the same way.

Iprepared Paranuclein from 20 eggs in the way described after 36 hours digestion with Pepsin. The Paranuclein was then dissolved in a weak ammoniacal solution. As solution took place very slowly in even large quantities of Ammonia-holding water(1500cc) , it was found necessary to leave the mixture over-night. Owing to the fact that the solution was fairly strongly ammoniacal, it was necessary to neutralise partly with ACetic Acid before precipitating with Alcohol. The body was then extracted with Alcohol at 60° and finally with Ether. This body after having been dried to constant weight at 105° and incinerated, was found to contain 2·88%P. -i.e. decidedly lower than that given by Bunge , this being in all probability due to the longer action of the Ammonia in the case of my preparation.

The Pepsin filtrate did not precipitate Syntonin.

Action of Trypsin on the Paranuclein of Ovovitellin.

1·397 gm. of this Paranuclein were digested with 80 cc Pancreatic infusion for 5 hours at 38° . About the half passed into solution . The undissolved remainder

Action of Trypsin on Hämatogen.

was washed with water acidified with acetic acid , then with distilled water, Alcohol and Ether and finally dried in exsiccator and at 105° to constant weight In this remainder the P. percentage was found to be 1.345%. The filtrate reacted alkaline but only gave a slight clouding on the addition of Acetic Acid, which ,however , on the addition of Albumoses or Syntonin was changed into a thick, white, flocculent precipitate . In order to see whether the Barium salt of this proteid-precipitating body could be prepared, I acidified the filtrate with a little Acetic acid , then added a cold saturated solution of Barium Hydrate until the mixture was slightly alkaline and precipitated with Alcohol . The watery solution of this precipitate had no visible effect on an Albumose solution.

Before drawing conclusions from this short study of the Paranuclein of Ovovitellin, I shall describe the results obtained from the other method.

The yolks of 20 eggs were extracted with Ether until completely colourless and then , after removal of the Ether, dissolved in 500 cc 10% Sodium Chloride. This was left over-night to ensure complete solution and then distilled water added until the precipitate of Vitellin had separated out . The precipitate was then redissolved in 5% NaCl and this in its turn separated out

by means of a large quantity of water . This was repeated 5 times and at the close a pure white precipitate was obtained . ~~In order to remove the Lecithin this was extracted with boiling Alcohol and then with Ether~~ . The Ovovitellin, obtained in this way , was dissolved in 0·25% HCl and digested with Pepsin for 24 hours at 38°. A white flocculent precipitate separated out in very small quantity . After filtration, this body was extracted with boiling Alcohol and then with Ether One portion was dried at 105° and ~~the~~ the P. estimated.

~~0·~~101 gm. gave 0·072gm. $Mg_2P_2O_7$

= 19·655% P.

The reason for this high P. percentage I cannot give. It may be due to decomposition from the action of Alcohol in the heat.

Another portion was digested for 12 hours longer and then purified in the way described above. It was found to contain 8·054%P.

This agrees with Salkowski's results obtained under the same conditions in the case of Casein Paranuclein with the exception that in the latter case a Proteid precipitating body was split off by peptic action. See page 75

The filtrate did not precipitate Syntonin nor Albumoses and its P. was almost entirely in organic form-0·0014% ortho- out of a total of0·0181 %.

Thess bodies were free from Lecithin and inorganic P. When I come to speak of the proteid precipitating body obtainable from the so-called Paranuclein of the ~~yellow~~ yolk of the egg, I shall refer to these rich Phosphorus holding bodies prepared by this second method from the Ovovitellin. The latter body with circa 8%

Phosphorus seems to be practically the same as that obtained by Altmann, while the body containing the very high percentage of P. almost seems to be a combination between an Anhydride of Phosphoric Acid and a small amount of very firmly attached proteid.

Bunge's Haematogen, be it a Paranuclein or a body of different nature, has most of the properties of the artificially prepared Paranuclein.

That is to say it is a combination between a rich P. holding organic Acid with Proteid detachable by Pepsin . Its P. percentage is a varying one. Bunge gave its percentage as rather over 5%P. This, however, as I have said before is not a fixed percentage . The longer it is subjected to the action of even very weak alkalies the lower does the P. percentage become. In all probability also other factors play a part eg. prolonged or brief action of Pepsin & acid alcohol subsequently may either when acting together or separately alter the P. percentage.

The body is split up by the action of Trypsin and alkalies, a proteid precipitating body being present among the soluble digestive products

In order to show the marked action of alkalies upon this Haematogen , i shall give here some analyses of the substance after prolonged action of weak ammoniacal solutions :-

Effect of prolonged action of weak alkalies on Haematogen

A quantity of Haematogen was prepared in the usual way by peptic digestion of the yolks of thirty eggs which had been previously extracted with Ether. The white flocculent precipitate which appeared after about 12 hours digestion was allowed to settle down completely and, in order to remove as far as possible any proteid matter that had been carried down with it, the digestion was allowed to continue for 48 hours. The body obtained in this way was immediately dissolved in about 500cc. of a weak ammoniacal solution and the mixture left overnight. Next morning Acetic Acid was added, and the white thick precipitate which at once appeared was allowed to settle completely. The supernatant fluid was siphoned off and the precipitate filtered until it was as far as possible dry. It was then finely divided up in a large quantity of Alcohol and allowed to stand in this for a few hours. It was in the next place filtered, and after complete removal of the Alcohol, extracted with Ether and dried afterwards in exsiccator. A small portion of this was dried at 105° to constant weight and the Phosphorus estimated by Weibull's method. It was found to contain 2·88%. The larger portion was again subjected to the action of Ammonia for 24 hours and the Haematogen reprecipitated by means of Acetic Acid. The precipitate was treated in the same way as the previous one and the Phosphorus estimated. Now it was found to contain 0·962%.

The Haematogen was then subjected to the action of Ammonia for the third time and the Phosphorus estimated as before. It was now found to contain only 0·711%.

That is to say, Ammonia and as I shall show later Sodium Hydrate, as well as Trypsin have the power of splitting ~~off~~ up the Haematogen leaving the residue gradually poorer in Phosphorus. ~~And~~ With regard to the nature of the body that is so split off, that is to say the rich Phosphorus holding Acid, I shall speak later. Here again there is a similarity betqeen the Nucleins and the so-called Para-Nucleins. It is of importance to note the marked action of alkalies on the Paranucleins especially as previously investigators have employed the method of repeated solution in alkalies to purify these bodies. The investigation of the alkaline solutions must go hand in hand with that of the precipitated body. The only one, ~~as~~ so far as my knowledge goes, who has referred to the action of the Pancreatic Juice on the Nucleo-Albumins (I use the term in Hammarsten's sense i.e. Pseudo-Nuclein plus Proteid removable by Pepsin) is Sebelien who tried the effect of the Trypsin on Casein, finding the latter was rapidly split up. He did not refer particularly to the nature of the bodies split off by the action of the ferment. No one, as far as my knowledge goes, has investigated the action of the Tryptic ferment on the so-called Paranuclein of Ovovitellin. One very important fact to be learned from the study of the artificial Para-nucleins is this that the acid component in the molecule

unities with a large amount of Proteid in such a way that on the combination being subjected to the action of Pepsin, the excess of Proteid is first of all removed and then later on the Paranuclein is gradually dissolved as such or rather through hydrolytic action transformed into Albumose-Paranucleins. It is only after extremely long digestion that the Paranuclein is split up by the ferment in such a way that its Phosphorus percentage is diminished. When we compare these results with those obtained from peptic digestion of the Pseudo- or Paranucleins as they exist in the milk or egg, we are struck by the close similarity. Here also, as for example in the Ovivitellin and the Casein, there is in the first place a large amount of Proteid in the molecule which is first of all removed by the Pepsin then the hydrolytic action takes place with splitting up into Albumose Pseudo- or Para-nucleins and only later does the undissolved residue begin to have the Phosphorus part split off into a body containing a larger percentage of Phosphorus than the Para- or Pseudo-nucleins.

When we remember also that the artificial Paranucleins are formed from a combination between Syntonin and a definite acid obtainable from the Nucleic Acid which in its turn is split off from the Nuclein of the Leucocytes and the Lymph cells of the Thymus gland, the closeness of the relationship between the socalled Para- or Pseudo-Nucleins and the true Nucleins of the Nuclei of the animal cell becomes strikingly impressed upon us.

Unfortunately our knowledge of this most intricate subject being still of a comparatively limited nature, our nomenclature can only be of a temporary nature.

I should like however to point out that when we at present speak of a Paranuclein we do not mean that the body contains the acid obtained by Kossel from the Nucleic acid of the Thymus and termed by him Paranucleic acid, but merely that they are bodies closely allied in many of their properties to the Nucleins. Hammarsten's term Pseudo-Nuclein is by no means of a happy nature and could only be used provisionally if used at all. There is no reason why they should be termed 'false' Nucleins. Hammarsten's objections to the word Paranuclein as opposed to Pseudo-nuclein are scarcely valid. Because in all probability there are numerous bodies included under the term which are of quite a different nature from the others, that is not to say that the term Paranuclein is inapplicable as it gives one the impression of <u>one</u> definite chemical body. There are in all probability a series of Nucleic Acids with different properties and yet we do not scruple to use the term Nuclein. For example the Nucleic Acid obtained from Yeast furnishes a Cupric Oxide reducing body on being heated with weak acid while the Nucleic Acid of Thymus does not, not to speak of the differences in the amounts and nature of the Bases obtainable from different Nucleic Acids. We have only to confess that the nomenclature is of a temporary nature and we must wait until our knowledge of the composition of these bodies is more complete before we can hope to attain to a perfect terminology.

The Proteid precipitating body of the ~~yellow~~ yolk of the Egg.

I now turn to the nature of the Phosphorusholding acid split off from the Paranuclein of Ovovitellinby the action of Alkalies. It is unnecessary to describe the method of preparation again, The Paranuclein (Haematogen) is prepared in the usual way, dissolved in a weak~~k~~ ammoniacal solution and this allowed to stand about 12 hours. The solution is then made strongly acid ~~by/kke~~ with Acetic Acid and the mixture filtered. Both the precipitate and the filtrate are to be preserved for further treatment.

1. In the first place the filtrate is poured into about twice its volume of Alcohol to which a small quantity of Ether has been added. A snow white finely flocculent precipitate falls slowly to the bottom of the vessel. In order to obtain this body proteid free, it is again dissolved in weak ammonia and precipitated out of its solution by Alcohol-Acetic Acid. The finely flocculent precipitate obtained in this way is then well washed with Alcohol and finally with Ether. After extraction three or four times at room temperature with the latter, the body tends to become slightly slimy. It is then placed in small glass dish and dried in exsiccator. After being completely dried it is rubbed up in a mortar to a fine white powder and then replaced in exsiccator.

2. The precipitate obtained on adding Acetic Acid in the first instance is dissolved in 2% Sodium Hydrate and the solution allowed to stand for some hours.

It ~~was~~ is then precipitated with Acetic Acid and the precipitate and filtrate both preserved.

The filtrate ~~was~~ is treated in the same way as the previous one (1)., and the precipitate in the same way as precipitate (2) The process was continued until the Acetic Acid precipitate was Phosphorus free or practically so. The body obtained from the original filtrate (1) was found to be of the same nature as that obtained in the way described under heading (2) so that one description suffices for both.

Properties of this proteid-precipitating body.

1. On boiling with dilute acids, it does not furnish Nuclein bases, therefore it is not a Nucleic Acid.

2. It is extremely easily soluble even in cold water, the watery solution reacting acid.

3. On acidifing this watery solution with Acetic acid, a slight white precipitate of the body at once appears. The same, even to a more marked extent, occurs on the addition of Hydrochloric Acid.

4. The solution has the property of precipitating Syntonin and Albumoses out of 2% Acetic acid solutions (also out of weaker acid solutions.). These precipitates (artificial Paranucleins) are easily soluble in weak Hydrochloric acid solutions, even in 0·25% acid, though not easily soluble in Acetic acid (if it be not over 2% strength)

5. It gives marked ~~Buiret~~ Biuret action, even more marked after the solution has been boiled for some time in Acetic acid and then filtered.

6. After boiling the watery or acidified solutions there is no transformation of the Phosphorus part of the molecule into the Ortho. form. That is to say there is no precipitation with Magnesia Mixture. Even after prolonged boiling, the solution still retains its property of precipitating Proteid and to as great an extent as previously.

7. Millon's reagent gives no sign of red colouration on heating with this body.

8. On the addition of Acetic acid and Ferrocyanide of Potash to the watery solution, no precipitation occurs.

9. A watery solution of the acid was tested with solutions of the following salts and gave results as follows:-

a	$Ca(oH)_2$	slight clouding
b	$Ca(Cl)_2$	" "
c	$MgSO_4$	Slight precipitate easily soluble in excess of the reagent
d	$HgCl_2$	No precipitate
e	H_2SO_4 (in excess) with Phosphortungstic Acid	White precipitate not easily soluble in excess of water, even on boiling.

(in the case of Paranucleic Acid out of the Nucleic Acid of Thymus the precipitate is exceedingly easily soluble in warm water)

9.cont.

f $AgNO_3$: precipitate easily soluble in excess of Ammonia or Nitric Acid.

g a watery solution does not reduce an ammoniacal solution of Siton Nitrate.

h $Pb(NO_3)_2$: a thick white insoluble precipitate

i $Ba(OH)_2$: no precipitate.

+ Alcohol = precipitation of the salt.

~~Quantitative~~

Quantitative.

The body was analysed five times in order to find out its Phosphorus percentage after repeated solution in weak Ammonia and reprecipitation by means of Alcohol and Acid. The following were the percentages obtained:-

1.	7·10%	Phosphorus
2	7·51%	"
3	7·51%	"
4	7·70%	"
5	7·94%	"

or 7·55% Phosphorus as average.

The Nitrogen was estimated by Kjeldahls process and was found to amount to:-

1	12·94%
2	12·42%

that is to say 12·68% Nitrogen as average.

Thus in this acid the atomic proportions are as

$$\frac{P}{7\cdot55}\ :\ \frac{N}{12\cdot68}$$

$$\frac{30\cdot96}{14\cdot01}$$

i.e. approximately

3·7 Nitrogen : 1 Phosphorus.

When we compare this with the atomic proportions between Phosphorus and Nitrogen in the Nucleic acid, we find a fairly close similarity.

In Nucleic Acid 3 N : 1 P (Kossel)

Both formulae given by Kossel for Nucleic Acids obtained from different sources contain the constant ratio N 3 : P 1 , thus, $C_{21}H_{49}N_9P_3O_{22}$ and $C_{30}H_{52}N_9P_3O_{17}$

I have to thank Professor Kossel for his great kindness in allowing me to give the formula of Paranucleic Acid from the Nucleic Acid of the Thymus , the analyses of which have not yet been published , namely:-

$C_{16}H_{25}N_3P_2O_{12}$, that is to say the ratio here is N. 1·5 : P. 1

I analysed also the Barium salt of Paranucleic Acid obtained by the method described on pages 79&80 and found the ratio to be N 1·6 : P 1 .

(The Barium salt was dried to constant weight in vacuum at a temperature of 80° in Schmiedeberg-Meyer's drying oven , as higher temperatures are apt to decompose the body and at atmospheric pressure it is impossible to dry the salt to constant weight under 105°).

Barium unites with Paranucleic Acid to form two salts - a neutral and a basic one . The neutral salt was evidently analysed by Kossel , while the ~~neutral~~ basic one was that analysed by me . I shall now give my reasons for regarding this as a definite acid of a different nature and composition from those discovered and described

by Kossel and Altmann.

1. Its Phosphorus percentage is lower than that of Nucleic Acid (which is about 10%) and decidedly lower than that of Paranucleic Acid (about 13%).
2. The ratio between Nitrogen and Phosphorus is different from either as above mentioned .
3. It is much more easily soluble than Nucleic Acid. Whether it is also more soluble than Paranucleic Acid we do not know as the latter has not yet been prepared in the free state , it having been analysed only in the form of its Barium salt
4. The precipitates which it causes with Syntonin in acetic acid solution are much more easily soluble in weak Hydrochloric acid than either those between Nucleic Acid or Paranucleic Acid and Syntonin are.
5. On boiling the watery solution , it does not furnish Nuclein Bases as Nucleic Acid does. In this it agrees with Paranucleic Acid.
6. It gives marked Biuret reaction. Neither Nucleic nor Paranucleic Acids do. This reaction is not due however to the presence of Proteid matter, but in all probability to the occurrence of an Amine holding body in a side chain , the radicle probably being an anhydride of orthophosphoric acid. I shall refer to this again .

7. The Phosphorus in its molecule does not seem to be so easily transformed into the ortho form as is the case with Nucleic or Paranucleic Acids

8. The precipitate which this body gives with Phosphortungstic Acid and Sulphuric Acid is by no means so easily soluble in warm water as that formed with Paranucleic Acid . The precipitate formed under like conditions with Nucleic Acid is only with difficulty soluble in warm water.

The acid has however some marked properties which make one inclined to group it with those of the Nuclein or Paranuclein series.

(1)). It has a high and constant percentage of Phosphorus in organic combination not due to the presence of Lecithin.

(2). It precipitates Proteids out of acetic acid solutions and this precipitating power is not of the nature of that possessed by a Metaphosphoric Acid because even after prolonged boiling this property is not absent nor is there any transformation into orthophosphoric acid.

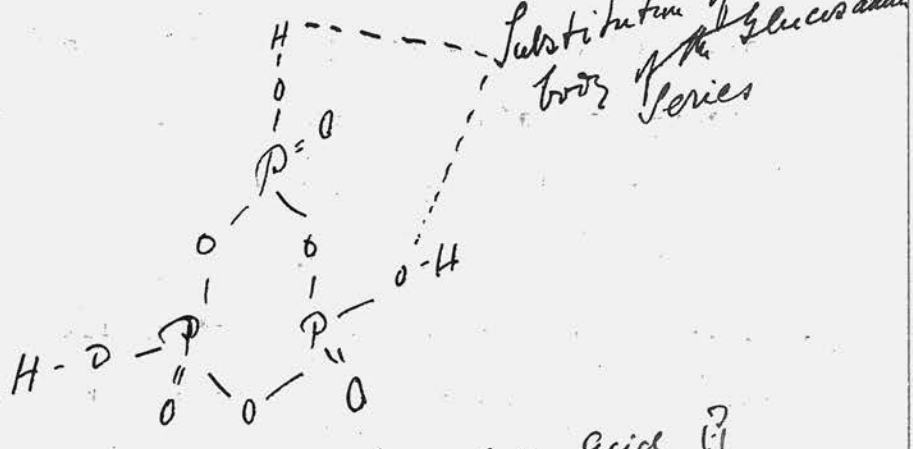
(3). It is prepared by the action of alkalies on bodies which possess almost every property of the artificially prepared Paranucleins . I hardly require to enter into the points of resemblance again,

except merely to recall the interesting fact that the Paranucleic Acid from the Nucleic Acid of Thymus has the property of combining with Syntonin in such a way that there is always a large amount of non-phosphorus holding proteid in the combination which is easily removable by pepsin. So also here in the Ovovitellin, one has a large amount of Albumin easily removable by pepsin, leaving after digestion a body closely allied to the artificial Paranuclein.

(4). The occurrence of such a body in the ~~yellow~~ yolk of the egg makes it almost certain that this rich Phosphorus holding acid plays a most important part as the forerunner of the true Nucleins of the nucleus of the adult cell.

That an anhydride of Phosphoric Acid plays a part in the Paranucleic Acid molecule is certain, but the nature of this anhydride is unknown. Liebermann regards it as a Monometaphosphoric Acid: in fact he regards the Nucleins as merely mixtures of this acid and precipitated Proteid with the Nuclein Bases as accompanying bodies, not in a state of combination. This theory does not now require more disproof as the preparation of definite acids such as Nucleic and Paranucleic Acids has rendered the theory an impossible one. I wish here merely to draw attention to the fact that the Trimetaphosphoric Acid (Fleitmann + Henneberg) salts have many points in common with the salts of the acid which I have described as occurring in yolk of egg.

(Ba salts ~~sol~~ sol. ~~sc~~ in cold & water)


Substitution of a
body of the Glucosamine
Series
Radicles of the Paranucleic acid (?)
+ Nucleic acid
+ Protein (perhaps identical with that of yolk of eggs)

Note. The Barium salts of Trimetaphosphoric Acid are easily soluble in water (according to Heitmann + Hemeberg) as are also those of Silver, Mercury, Potassium + Sodium. The salts of Paranucleic acid agree in these respects with the corresponding ones of this polymer of meta-phosphoric acid. When we examine closely the formula of Paranucleic acid + remember at the same time the constancy of the protein precipitating property in this as in the other acids of the Nucleic + Paranucleic series, the possibility or rather probability of the presence of a polymer of Metaphosphoric acid, and especially of a Trimetaphosphoric acid becomes strengthened. The nature of the body in the side chain, we can only guess at. It is in all probability a body of the nature of Glucosamin from the decomposition products obtained from nucleic acid viz. Lavulinic acid, Ammonia, Formic acid.

In the formation of a Nuclein or a Paranuclein is there a saturation of the free acid affinities?

Before I group together under headings the conclusions to be drawn from the preceding investigations, I wish to give the results obtained from the estimation of the acidimetric values of the components of the true Nucleins and Paranucleins before and after ~~esterification~~ combination. The question, asto whether the formation of a Nuclein, or a Paranuclein is accompanied by saturation of the acid affinities or not, may be answered in this way.

Example I

1. The acidity of the Syntonin Solution has to be in the first place estimated against 1/20 Norm. NaOH.
2. In the same way the acidity of the Nucleic Acid solution is estimated.
3. Both are mixed together, excessof Syntonin being added to take up all the Nucleic Acid.
4. The Nuclein formed in this way is dissolved in a known quantity of NaOH (1/20), excess of the latter being used.
5. The excess of Soda is titrated against 1/20 Norm. Oxalic Acid.

In this way from (1)and (2) we learn the acidimetric values of the free acids and from that we can calculate the Values of the acid solutions used in (3) to form the Nuclein. Here all is estimated in the free state.

From (4) minus (5) we obtain the acidimetric value of the combination and we can then see whether the latter is lower than the value of the acids before combination, the difference giving the amount that has been taken up.

I shall give one from a series of estimations:-

A. 2,043g Nucleic Acid were dissolved in 200cc. Water

49cc. of this solution = 29·59cc. ½₀Norm NaOH.

B. Syntonin solution in 0·25% HCl

of which 20cc. = 23·6cc. ½₀Norm. NaOH.

C. 49·4cc. Syntonin HCl were precipitated by 49cc. of the Nucleic Acid solution i.e. stated interms of NaOH ½₀ Norm:-

49·4cc. Syntonin = 58·29cc. NaOH ½₀Norm.

49cc. Nucleic Acid solution = 29·59cc. NaOH ½₀Norm.

reckoned free = 87·88cc. NaOH ½₀ Norm.

D. The excess of Syntonin used was titrated against NaOH ½₀Norm. 54·05cc. NaOH ½₀N were necessary for this purpose i.e. 87·88-54·05 = 33·83cc. the value in terms of NaOH ½₀N of the free acids which had gone to form the Artificial Nuclein.

E. The precipitate (Nuclein) was dissolved completely by 84·6cc. ½₀N. NaOH.

F. Excess of NaOH ½₀N was titrated against ½₀N. Oxalic Acid 53·1cc. of the latter were required i.e.:-

84·6-53·1 = 31·5cc. NaOH = the value of the Acids after combination in terms of NaOH ½₀N.

Before combination = 33·83 NaOH ½₀ Norm.

After " = 31·50

2·35cc.

2·35cc. ½₀N. NaOH = 3·89cc. Nucleic acid solution, or 3·89cc. Nucleic acid have been saturated by the

combination with Syntonin. Here owing to the fact that the NaOH was allowed to act on the combination so long (in E) that in all probability there was a decomposition with a setting free of the free Nucleic Acid. In order to avoid this, I carried out the experiment again, taking precautions not to allow the Sodium Hydrate to act long upon the Nuclein. I shall give the results as shortly as possible here.

Example 11

(1) Syntonin HCl Solution.

50cc. Syntonin = 51·6cc. NaOH 1/20N.

(2) Nucleic Acid Solution
(1·01g. in 76cc. water)

20cc. = 13·3cc. NaOH 1/20N.

(3) Mixture of both

250·8cc. Syntonin were precipitated by 56cc. Nucleic acid solution.

	NaOH 1/20N.
ie. Syntonin:- 250·8cc.	= 258·8cc
plus Nucleic Acid:-5·6cc.	= 32·2cc.
Syntonin & Nucleic Acid	= 291·0cc.
minus excess of Syntonin	= 188·4cc.
ie. free reckoned SyntoninHCl & Nucleic Acid	= 102·6cc. NaOH 1/20N.

(4) 194·25cc. NaOH 1/20N. were required to dissolve the precipitate completely i.e. in excess of the Sodium Hydrate.

(5)

Excess of NaOH titrated against N. Oxalic Acid

130 4cc. Oxalic acid were required

ie. 194·25
-130·40
63·85cc. NaOH ($\frac{1}{20}$N) the value in terms of Soda of the components after combination

102·6 free reckoned
- 63·85 combined
38·75cc. Nucleic Acid solution in terms of NaOH ($\frac{1}{20}$N)

saturated during combination with the Syntonin ie. rather more than 6cc. NaOH $\frac{1}{20}$ N. more than the value in terms of Soda of the original Nucleic Acid. That is to say in this latter case, more than the acid affinities of the original Nucleic Acid have been saturated.

This may be due to the fact of the transformation of an acid globulin such as Syntonin (which reacts alkaline) into an acid reacting Nuclein.

In excess of Syntonin also, the Nucleic acid may combine with 2 or more equivalents of the acid globulin*.

In the case of the synthetically prepared Paranucleins, I have employed the same method ie. estimation of the values (in terms of $\frac{1}{20}$ Norm NaOH) of the free acid solutions and of the same after combination. As with the Nucleins, so also here there is a saturation in part of the acid affinities of the two components, but owing to the fact that $\frac{1}{20}$ Norm. NaOH so readily decomposes the artificial Para-Nucleins, it was found impossible to obtain results of a constant character.

And as it was necessary to employ ½ Norm Sodium Hydrate solution in order to dissolve the Paranuclein properly I could not carry out the method with weaker solutions. What is certain is this that with the formation of a Nuclein or a Paranuclein there is saturation at least partially of the acid affinities of the constituent components.

Conclusions drawn from the results given in the ~~foregoing~~ foregoing pages.

1. The synthetically prepared Nucleins agree practically in every respect with those occurring naturally in the Nuclei of different ~~animal~~ cells in the animal body. The points of resemblance have been all fully described before and do not require to be mentioned here.
2. All the Nucleins which I have examined have a fairly constant Phosphorus percentage, which is only slightly affected even after very prolonged action of Pepsin. The Peptic ferment, however gradually dissolves the Nuclein, not however lowering to any appreciable extent the Phosphorus percentage in the undissolved remainder.
3. They are all (synthetically prepared and natural) easily decomposed both by weak alkaline solutions and by Trypsin.
4. In only one of the number was the combination between Nucleic acid and Proteid a loose one ie. Thymus Nuclein.

5. In only one, was a proteid precipitating body split off by the action of Pepsin viz. in the case of Pancreas Nucleins

6. With one exception, Trypsin & 0 25% Na_2CO_3 always split off a proteid precipitating body from the Nuclein after prolonged digestion; that exception is the Nuclein of the red blood corpuscles of the goose and in all pro-babability also those of hen and duck.

7. The Phosphorus in the soluble digestive products is always, after Peptic as after Tryptic and alkaline digestion, almost entirely in organic form.
There is only one exception to this and that is the case of the Nuclein of the red blood corpuscles of the hen & duck.

8. The synthetically prepared Albumose Nucleins retain many of the properties of the Albumoses e.g. physical properties, solubilities etc.

9. The synthetically prepared Paranucleins agree in almost every respect with the natural Paranucleins - their solubilities, decompositions, percentage of Phosphorus etc.

10. The synthetically prepared and natural Paranucleins are slowly dissolved by Pepsin. After very prolonged action they are partly decomposed by the same ferment.

11. All the Paranucleins examined were easily decomposed by Trypsin and Alkalies, a proteid precipitating body being split off.

12. In the yolk of the egg there is a rich Phosphorus holding acid present, the nature of which has not previousl been properly recognised. For the nature of this body

and its relationship to the Nucleic and Paranucleic acids, I must refer to the description given in the paper.

13. In the combination between Nucleic Acid and Syntonin, there is at least partial saturation of the acid affinities of the constituent components.
The same is true with regard to the Syntonin Paranuclein.

In grouping together the conclusions to be drawn from my investigations on the nature of the Nucleins, Paranucleins and the proteid precipitating body in the yolk of the egg, I have confined myself entirely to my own results. I have tried, in the first place, to give as far as possible a short and succinct account of our present day knowledge of the Nucleins, and Paranucleins and allied bodies occurring in the cell nucleus or protoplasm.
Although the whole subject has only come into prominence within the last few years, a mass of literature has accumulated to such an extent that a mere descriptive account of the work of other investigators would require quite as long a paper as the present one to give full justice to the subject. So I was compelled to restrict myself largely to my own work, with the exception of the introductory chapter on the chemistry of the cell nucleus.
Notwithstanding, at all points where work has been done touching on that carried out by myself, I have not failed to mention the nature of that work with the names of author and title of paper. The references to Literature

are as nearly as possible complete. In some cases papers have been referred to which give the older references more completely than my own.

I have been occupied at this research practically every day and all day for more than twelve months. If I have in any way helped to advance our knowledge of the intensely interesting subject, the chemistry of the animal cell my labour will have been more than amply repaid.

I have to thank among many others, my revered teacher, Prof. Kossel of the Physiological Institute, Marburg, for his never varying kindness and assistance--

and Prof. Rutherford for the courteous way in which he put his laboratory and library at my disposal since my arrival in Scotland.

I have been greatly assisted in the carrying out of this research by a grant of £30 from the Dickson Travelling Fund of this University.

Note: Unlike the Nucleo-Proteid of the Pancreas, the Nuclein of the red blood corpuscles of the Goose does not furnish a cupric oxide reducing body even after prolonged action by weak acid. I have subjected varying quantities of the Nuclein to the action of 0.2% HCl for from 1 to 15 hours at temperatures varying from 15° to 100°C. I then decolorised the solution by means of charcoal and tested the colourless filtrate (after having been made strongly alkaline) against Fehling's solution. In no case did I obtain reduction.

Table II

The Form in which the Phosphorus is present in the digestive fluids after digestion of the Nucleins and Paranucleins

a. = inorganic Phosphates
b. = organic " } %

Nuclein etc. employed	after action of Pepsin.	after action of Trypsin.	after action of $NaCl_3$ (0.2%)	
Leontorin-Nuclein	(a) 7.799 % (b) 92.201 %	(a) 17.143 % (after 17 hours' digestion) (b) 82.857 %	(a) 39.453 % (b) 60.546 %	I have given only single or at most double examples under each Nuclein as the table is only given as an illustration of the method in which the digestive ferments act.
"	(a) 8.013 % (b) 91.987 %	(a) 15.172 % (after 12 hours' digestion) (b) 84.828 %	(a) 28.000 % (b) 72.000 %	
Nuclein (Duck)	(a) 42.858 % (b) 57.142 %	(a) 47.223 (b) 52.777	———	
" (Goose)	(a) 15.71 % (b) 84.29 %	(a) 6.10 % (b) 93.90 %	———	
" (Thymus)	(a) 8.678 % (b) 91.322 %	(a) 8.373 % (after 4 hrs. digestion) (b) 91.627 %	(a) 4.737 (after 4 hours' digestion) (b) 95.263	
" (Pancreas)	(a) 3.18 % (b) 96.82 %	(a) 23.53 % (b) 76.47 %	———	The same holds for Table I
Paranuclein (Coccoid-ellin)	(a) 7.73 % (b) 92.27 %	. . .		
Leontorin-Paranuclein	(a) 8.00 % (b) 92.00 %	(a) 3.93 % (b) 96.07 %		

With regard to duration of digestion process & amount of digestive agent used in each case in the different tests see the paper.

Table I. The Phosphorus Percentage of Nucleins and Paranucleins.

Nucleins examined	Preparation Pure	after action of Pepsin	after action of Pancreatic Infusion	after action of 0,2 5.% Na_2CO_3
Lyntonin-Nuclein	3·490 %	3·859 %	1·469 %	1·998 %
"	3·494 %	3·679 %	0·993 %	0·653 %
Deutero-albumose Nuclein	6·320 %	6·290 %	—	—
albumose-Nuclein (Witte's Pepton)	3·535 %	5·426 %	—	—
	5·914 %	5·809 %	—	—
Nuclein (Ducks' blood)	4·248 %	—	—	—
" (Seagull blood)	4·301 %	—	—	—
" (after Kossel)	6·010 %	5·6 %	—	—
" of Thymus	4·416 %	4·325 %	1·838 % (digested 4 hours)	2·503 % (digested 4 hours)
Nucleo-Protein of Pancreas	2·796 %	3·196 %	1·120 %	
Paranuclein of Yolk of egg	3·19 (Bunge	varies largely	1·345 %	
Lyntonin-Paranuclein	2·755 %	5·045 %	1·584 %	

Literature.

Nuclein

Lubavin, Med. - chem. Untersuch. von. Hoppe-Seyler. Heft. 4. p. 463

Miescher, the same Heft 4. p. 441&502

" Verhand. der naturforsch. Gesell. zu Basel Bd. 6. p. 138

Nucleic Acid

Altmann, Du Bois Reymond's Archiv. phys. Abtheil. 1889 p. 525.

Kossel, " " " 1891 p. 181

" " " " 1890 (Verhand. der phys. Gesell. zu Berlin 6Feb.)

" " " " " 1893 p. 157-164

" Ber. der deutsch. chem. GESELL. Bd26 p2753 also 27/p.2215

" Sitzungsbericht. d. Berliner Akad. d. Wissenschaft Bd 18. 1894

" Verhalten des Nucleins des Hühner Eidotter's bei der Bebrut. Zeit. fur phys. Chem. 10. p. 248

" Gehalt der Muskelnan an Nuclein 10. p. 268

" Centralbl. f. d. med. Wissensch. 1893 p. 497

" &Lilienfeld on Nucleo-Histon Zeit. f. phys. Chem. Bd. 18

Artificial Nucleins or Combinations of Proteids with HPO_3

Liebermann, Pflüger's Archiv. Bd. 47&Centralbl. f. d. med. Wissensch. 1893p. 465&737

Malfatti, Zeit. f. phys. Chem. Bd. 16. p. 68. 73&75

Pohl, " " " 13 p. 293-296

Digestion of Nucleins + Paranucleins

Bokay, Zeit. f. P. Chem. Bd. 1p. 157

Popoff, " " " 18 p. 533

Sebelien, " " " Bd. 20

Gumlich, " " " " "

Weintraud, Verhand. d. p. GESELL. zuBerlin 1895

Salkowski&Hahn, Pflüger's Archiv. Bd. 59

Willdenow, (Drechsel's Laboratory) Dissert. Bern 1893

Moraczewski, Zeit. f. p. Chem. 1895

Sandmeyer, " " " 1895

Literature (cont.).

Reparation of albumoses

Kühne & Chittenden Zeit. f. Biol. Bd. 20 p11
" " " " " " 22 p. 435
Neumeister " " " 23 p. 381

Fleischsäure + Phosphor-fleischsäure

Siegfried Ber. d. k. sächs. Gesell. d. Wissensch. zu Leipzig Math. phys. Classe 1893 p. 485-489
" Ber. d. deutsch. chem. Gesell. Bd. 28
" Archiv. f. Anat. u. Phys. phys. Abtheil. 1894

Ichthyulin

Walther Zeit. f. p. Chem. Bd. 15 p. 477
Vaenciennes & Fremy Compt. Rend. T. 38 p. 471
Gobley Journ. de pharm. et de chim. III série T. 17 p401

Haematogen → Bunge Zeit. f. p. Chem. Bd. 9 p. 49

Paranuclein of Casein → Willdenow, Salkowski & Hahn, Moraczewski loc. cit.

Nuclein of Pancreas → Hammarsten Zeit. f. p. Chem. Bd. 19-

" " Birds' red corpuscles Kossel " " " Bd. 8 p-511
Lilienfeld & Verhand. d. p. Ges. zu Berlin 1891-2 No. 11&16

Fleitmann & Henneberg Liebig's Annalen Bd. 65 p. 304

(Trimetaphosphoric acid + its salts also the other polymers of HPO_3)

Effects of different staining reagents on different parts of cell during growth etc.

Zacharias, Uber Beziehenheit des Zellenwachsthums zur Beschaffenheit des Zellkerns. Ber. d. deutsch. Botanisch. ~~Chem~~ Ges. 1894 Bd. 12. Heft 5.
(references to the rest of his papers given there)

Lilienfeld, Uber die Wahlverwandschaft der Zellelemente zu gewissen Farbstoffen. Du Bois-Reymond's Archiv. - Verh. d. p. Gesell. zu Berlin 1893 p. 391.

Literature (cont.).

Halliburton	Goulstonian Lectures	1893
"	Brit. Med. Journ.	1893.
"	Journ. of Physiology	1893
"	"	1894 etc.
Weibull	Chemiker Zeitung	1892.
Heine	Zeit. fur Phys. Chem. Heft 5&6 Bd. 21.	1896.
Siegfried	" " " " " " "	

scattered papers on Nucleo-Albumins.

Third Destination P.

Clinical micro-chemical reactions

Nucleinsäure

I hereby certify that the thesis has been entirely composed by myself.

J. H. Libby.

Additional Note

Freshly prepared Fibrin has the power of fixing Nucleic acid & withdrawing it entirely from its solutions. Even after prolonged action of Pepsin the combination between Fibrin & Nucleic acid ("Fibrin-Nuclein") the Phosphorus percentage shows hardly any alteration.

The Phosphorus percentage varies from 2·86 - 3%. The importance of this property of the Fibrin is self evident.

Professor Simpson

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